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(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT AND DIAGNOSIS OF IMMUNE DISORDERS

(57) Abstract: The present invention relates to methods and compositions for the treatment and diagnosis of immune disorders, especially T helper lymphocyte-related disorders, and also for the treatment of mast cell-related processes and disorders, ischemic disorders and injuries, including ischemic renal disorders and injuries. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via the ability of their gene products to interact with gene products involved in the differentiation, maintenance and effector function of such TH cells and TH cell subpopulations. The genes identified can be used diagnostically or as targets for therapeutic intervention. In this regard, the present invention provides methods for the identification and therapeutic use of compounds as treatments of immune disorders, especially TH cell subpopulation-related disorders. Additionally, methods are provided for the diagnostic evaluation and prognosis of TH cell subpopulation-related disorders, for the identification of subjects exhibiting a predisposition to such conditions, for monitoring patients undergoing clinical evaluation for the treatment of such disorders, and for monitoring the efficacy of compounds used in clinical trials. Methods are also provided for the treatment of symptoms associated with mast cell-related processes or disorders and ischemic disorders and injuries using the genes, gene products and antibodies of the invention.

COMPOSITIONS AND METHODS FOR THE TREATMENT AND DIAGNOSIS OF IMMUNE DISORDERS

This is a continuation-in-part of U.S. patent application Serial No. 09/032,337, filed February 27, 1998, which is a continuation-in-part of U.S. patent application Serial No. 08/609,583, filed March 1, 1996, which is a continuation-in-part of U.S. patent application Serial No. 08/487,748, filed June 7, 1995, now U.S. Patent No. 5,721,351, which is a continuation-in-part of U.S. patent application Serial No. 08/398,633, filed March 3, 1995, each of which is incorporated herein by reference in its entirety.

1. INTRODUCTION

The present invention relates to methods and compositions for the treatment and diagnosis of immune disorders, especially T lymphocyte-related disorders, including, but not limited to, chronic inflammatory diseases and disorders, such as Crohn's disease, reactive arthritis, including Lyme disease, insulin-dependent diabetes, organ-specific autoimmunity, including multiple sclerosis, Hashimoto's thyroiditis and Grave's disease, contact dermatitis, psoriasis, graft rejection, graft versus host disease, sarcoidosis, atopic conditions, such as asthma and allergy, including allergic rhinitis, gastrointestinal allergies, including food allergies, eosinophilia, conjunctivitis, glomerular nephritis, certain pathogen susceptibilities such as helminthic (e.g., leishmaniasis) and certain viral infections, including HIV, and bacterial infections, including tuberculosis and lepromatous leprosy. For example, genes which are differentially expressed within and among T helper (TH) cells and TH cell subpopulations, which include, but are not limited to TH0, TH1 and TH2 cell subpopulations are identified. Genes are also identified via

the ability of their gene products to interact with gene
products involved in the differentiation, maintenance and
effector function of such TH cells and TH cell
subpopulations. Among the genes identified are ones involved
5 in repair or recovery of tissue from ischemic disorders or
injuries. The genes identified can be used diagnostically or
as targets for therapeutic intervention. In this regard, the
present invention provides methods for the identification and
therapeutic use of compounds as treatments of immune
10 disorders, especially TH cell subpopulation-related
disorders. The present invention also provides methods for
treating ischemic disorders or injuries. Additionally,
methods are provided for the diagnostic evaluation and
prognosis of TH cell subpopulation-related disorders, for the
15 identification of subjects exhibiting a predisposition to
such conditions, for monitoring patients undergoing clinical
evaluation for the treatment of such disorders, and for
monitoring the efficacy of compounds used in clinical trials.

20   2. BACKGROUND OF THE INVENTION
        Two distinct types of T lymphocytes are recognized:
CD8· cytotoxic T lymphocytes (CTLs) and CD4· helper T
lymphocytes (TH cells). CTLs recognize and kill cells which
display foreign antigens of their surfaces. CTL precursors
25 display T cell receptors that recognize processed peptides
derived from foreign proteins, in conjunction with class I
MHC molecules, on other cell surfaces. This recognition
process triggers the activation, maturation and proliferation
of the precursor CTLs, resulting in CTL clones capable of
30 destroying the cells exhibiting the antigens recognized as
foreign.
        TH cells are involved in both humoral and cell-mediated
forms of effector immune responses. With respect to the
humoral, or antibody, immune response, antibodies are
35 produced by B lymphocytes through interactions with TH cells.
Specifically, extracellular antigens are endocytosed by
antigen-presenting cells (APCs), processed, and presented

preferentially in association with class II major
histocompatibility complex (MHC) molecules to CD4⁺ class II
MHC-restricted TH cells. These TH cells in turn activate B
lymphocytes, resulting in antibody production.

5   The cell-mediated, or cellular, immune response,
functions to neutralize microbes which inhabit intracellular
locations. Foreign antigens, such as, for example, viral
antigens, are synthesized within infected cells and presented
on the surfaces of such cells in association with class I MHC
10 molecules. This, then, leads to the stimulation of the CD8⁺
class I MHC-restricted CTLs.

        Some agents, such as mycobacteria, which cause
tuberculosis and leprosy, are engulfed by macrophages and
processed in vacuoles containing proteolytic enzymes and
15 other toxic substances. While these macrophage components
are capable of killing and digesting most microbes, agents
such as mycobacteria survive and multiply. The agents'
antigens are processed, though, by the macrophages and
presented preferentially in association with class II MHC
20 molecules to CD4⁺ class II MHC-restricted TH cells, which
become stimulated to secrete interferon-γ, which, in turn,
activates macrophages. Such activation results in the cells'
exhibiting increased bacteriocidal ability.

        TH cells are composed of at least two distinct
25 subpopulations, termed TH1 and TH2 cell subpopulations.
Evidence suggests that TH1 and TH2 subtypes represent
extremely polarized populations of TH cells. While such
subpopulations were originally discovered in murine systems
(reviewed in Mosmann, T.R. and Coffman, R.L., 1989, Ann. Rev.
30 Immunol. 7:145), the existence of TH1- and TH2-like
subpopulations has also been established in humans (Del
Prete, A.F. et al., 1991, J. Clin. Invest. 88:346; Wiernenga,
E.A. et al., 1990, J. Imm. 144:4651; Yamamura, M. et al.,
1991, Science 254:277; Robinson, D. et al., 1993, J. Allergy
35 Clin. Imm. 92:313). While TH1-like and TH2-like cells can
represent the most extremely polarized TH cell
subpopulations, other TH cell subpopulations, such as TH0

cells (Firestein, G.S. et al., 1989, J. Imm. 143:518), which
represent TH cells which have characteristics of TH1 and TH2
cell subpopulations.

        TH1-like and TH2-like cells appear to function as part
5 of the different effector functions of the immune system
(Mosmann, T.R. and Coffmann, R.L., 1989, Ann. Rev. Imm.
7:145). Specifically, TH1-like cells direct the development
of cell-mediated immunity, triggering phagocyte-mediated host
defenses, and are associated with delayed hypersensitivity.
10 Accordingly, infections with intracellular microbes tend to
induce TH1-type responses. TH2 cells drive humoral immune
responses, which are associated with, for example, defenses
against certain helminthic parasites, and are involved in
antibody and allergic responses.

15   It has been noted that the ability of the different TH
cell types to drive different immune effector responses is
due to the exclusive combinations of cytokines which are
expressed within a particular TH cell subpopulation. For
example, TH1 cells are known to secrete interleukin-2 (IL-2),
20 interferon-γ (IFN-γ), and lymphotoxin, while TH2 cells
secrete interleukin-4 (IL-4), interleukin-5 (IL-5), and
interleukin-10 (IL-10).

        It is thought that TH1 and TH2 subpopulations arise from
a common naive precursor (referred to as THP). For example,
25 naive CD4⁺ cells from mice which express a single transgenic
T cell receptor can be induced to develop into either the TH1
or TH2 cell type. The conditions of antigen stimulation,
including the nature and amount of antigen involved, the type
of antigen-presenting cells, and the type of hormone and
30 cytokine molecules present seem to all represent determinants
of the pattern of TH1 versus TH2 differentiation, with,
perhaps, the decisive role belonging to the cytokines
present. With such a complex series of possible
determinants, a full accounting of the exact factors
35 important in driving TH1 or TH2 differentiation are, as yet
largely unknown.

Further, it has recently been noted that, in addition to CD4⁺ TH cells, CD8⁺ CTLs can, under certain conditions, also exhibit TH1-like or TH2-like cytokine profiles (Seder, R.A. et al., 1995, J. Exp. Med. 181:5-7; Manetti, R. et al., 1994,
5 J. Exp. Med. 180:2407-2411; Maggi, E. et al., 1994, J. Exp. Med. 180:489-495). While the precise functional role of such CD8⁺ TH-like cells is currently unknown, these cell subpopulations appear to have great relevance to immune responses against infectious agents such as viruses and
10 intracellular parasites.

Once TH1 and TH2 subpopulations are expanded, the cell types tend to negatively regulate one another through the actions of cytokines unique to each. For example, TH1-produced IFN-γ negatively regulates TH2 cells, while TH2-
15 produced IL-10 negatively regulates TH1 cells. Moreover, cytokines produced by TH1 and TH2 antagonize the effector functions of one another (Mosmann, T.R. and Moore, 1991, Immunol. Today 12:49).

Failure to control or resolve an infectious process
20 often results from an inappropriate, rather than an insufficient immune response, and can underlie a variety of distinct immunological disorders. Such disorders can include, for example, atopic conditions (i.e., IgE-mediated allergic conditions) such as asthma, allergy, including
25 allergic rhinitis, dermatitis, including psoriasis, pathogen susceptibilities, chronic inflammatory disease, organ-specific autoimmunity, graft rejection and graft versus host disease. For example, nonhealing forms of human and murine leishmaniasis result from strong but counterproductive TH2-
30 like-dominated immune responses. Lepromatous leprosy also appears to feature a prevalent, but inappropriate, TH2-like response.

It is possible that another example can be HIV infection. Here, it has been suggested that a drop in the
35 ratio of TH1-like cells to other TH cell subpopulations can play a critical role in the progression toward disease symptoms. Further, it has been noted that, at least in

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vitro, TH2-like clones appear to be more efficient supporters
of HIV viral replication than TH1-like clones.

Further, while TH1-mediated inflammatory responses to
many pathogenic microorganisms are beneficial, such responses
5 to self antigens are usually deleterious. It has been
suggested that the preferential activation of TH1-like
responses is central to the pathogenesis of such human
inflammatory autoimmune diseases as multiple sclerosis and
insulin-dependent diabetes. For example, TH1-type cytokines
10 predominate in the cerebrospinal fluid of patients with
multiple sclerosis, pancreases of insulin-dependent diabetes
patients, thyroid glands of Hashimoto's thyroiditis, and gut
of Crohn's disease patients, suggesting that such patients
mount a TH1-like, not a TH2-like, response to the antigen(s)
15 involved in the etiopathogenesis of such disorders.

A primary goal, for both diagnostic and therapeutic
reasons, therefore, would be the ability to identify, isolate
and/or target members of a particular TH cell subpopulation.
The ability to identify those genes which are differentially
20 expressed within and/or among such TH cell subpopulations is
required to achieve such a goal. To date, investigations
have focused on the expression of a limited number of
specific known cytokines and cytokine receptors in the TH
cell population. Cytokines, however, exert effects on cell
25 types in addition to specific TH cell subpopulations, i.e.,
exhibit a variety of pleiotropic effects. It would be
beneficial, therefore, to identify reliable markers (e.g.,
gene sequences) of TH cell subpopulations whose effects are
TH cell subpopulation specific, e.g., which, unlike secreted
30 cytokines, are TH cell subpopulation specific.

3.   SUMMARY OF THE INVENTION

The present invention relates to methods and
compositions for the treatment of immune disorders,
35 especially T helper (TH) cell and TH cell-like related
disorders. The present invention additionally relates to
methods and compositions for treating, ameliorating or

modulating ischemic disorders or injuries or mast cell-
related processes or disorders.

First, genes are identified and described which are
differentially expressed within and among TH cells and TH
5 cell subpopulations. Second, genes are identified and
described which are differentially expressed within TH cell
subpopulations in TH cell subpopulation-related disorders.
The modulation of the expression of the identified genes
and/or the activity of the identified gene products can be
10 utilized therapeutically to ameliorate immune disorder
symptoms and to modulate TH cell responsiveness, for example,
responsiveness to antigen. Further, the identified genes
and/or gene products can be used to diagnose individuals
exhibiting or predisposed to such immune disorders. Still
15 further, the identified genes and/or gene products can be
used to detect TH cell responsiveness, for example,
responsiveness to antigen.

"Differential expression," as used herein, refers to
both quantitative as well as qualitative differences in the
20 genes' temporal and/or cellular expression patterns within
and among the TH cell subpopulations. Differentially
expressed genes can represent "fingerprint genes" and/or
"target genes".

"Fingerprint gene," as used herein, refers to a
25 differentially expressed gene whose expression pattern can be
utilized as part of a prognostic or diagnostic evaluation of
immune disorders, e.g., TH cell-related disorders, or which,
alternatively, can be used in methods for identifying
compounds useful in the treatment of such disorders. For
30 example, the effect of the compound on the fingerprint gene
expression normally displayed in connection with the disorder
can be used to evaluate the efficacy of the compound as a
treatment for such a disorder, or may, additionally, be used
to monitor patients undergoing clinical evaluation for the
35 treatment of such disorders.

"Fingerprint pattern," as used herein, refers to the
pattern generated when the expression pattern of a series

(which can range from two up to all the fingerprint genes
which exist for a given state) of fingerprint genes is
determined. A fingerprint pattern can be used in the same
diagnostic, prognostic, and compound identification methods
5 as the expression of a single fingerprint gene.

"Target gene," as used herein, refers to a
differentially expressed gene involved in immune disorders,
e.g., TH cell related disorders, such that modulation of the
level of target gene expression or of a target gene product
10 activity can act to ameliorate the immune disorder.
Compounds that modulate target gene expression or activity of
the target gene product can be used in the treatment of
immune disorders.

Further, "pathway genes" are defined via the ability of
15 their gene products to interact with gene products involved
in TH cell subpopulation-related disorders and/or to interact
with gene products which are involved in the differentiation
and effector function of the TH cell subpopulations. Pathway
genes can also exhibit target gene and/or fingerprint gene
20 characteristics.

Although the target, fingerprint and/or pathway genes
described herein can be differentially expressed within
and/or among TH cell subpopulations, and/or can interact with
TH cell subpopulation gene products, the genes can also be
25 involved in mechanisms important to additional immune
processes.

The invention encompasses the following nucleotides,
host cells expressing such nucleotides and the expression
products of such nucleotides: (a) nucleotides that encode a
30 mammalian differentially expressed and/or pathway gene
product including, but not limited to a human and murine 10,
54, 57, 105, 106, 161 and 200 gene product; (b) nucleotides
that encode portions of a differentially expressed and/or
pathway gene product that corresponds to its functional
35 domains, and the polypeptide products encoded by such
nucleotide sequences, and in which, in the case of receptor-
type gene products, such domains include, but are not limited

to extracellular domains (ECD), transmembrane domains (TM)
and cytoplasmic domains (CD); (c) nucleotides that encode
mutants of a differentially expressed and/or pathway gene
product, in which all or part of one of its domains is
5 deleted or altered, and which, in the case of receptor-type
gene products, such mutants include, but are not limited to,
soluble receptors in which all or a portion of the TM is
deleted, and nonfunctional receptors in which all or a
portion of CD is deleted; and (d) nucleotides that encode
10 fusion proteins containing a differentially expressed and/or
pathway gene product or one of its domains fused to another
polypeptide.

The present invention also includes the products of such
fingerprint, target, and pathway genes, as well as antibodies
15 to such gene products. Furthermore, the engineering and use
of cell- and animal-based models of TH cell subpopulation-
related disorders to which such gene products can contribute,
are also described.

The present invention also relates to methods for
20 prognostic and diagnostic evaluation of various TH cell
subpopulation-related disorders, and for the identification
of subjects who are predisposed to such disorders.
Furthermore, the invention provides methods for evaluating
the efficacy of drugs for immune disorders, and monitoring
25 the progress of patients involved in clinical trials for the
treatment of such disorders.

The TH cell subpopulation-related disorders described
herein can include, for example, TH1 or TH1-like related
disorders or can, alternatively, include TH2 or TH2-like
30 related disorders. Examples of TH1 or TH1-like related
disorders include chronic inflammatory diseases and
disorders, such as Crohn's disease, reactive arthritis,
including Lyme disease, insulin-dependent diabetes, organ-
specific autoimmunity, including multiple sclerosis,
35 Hashimoto's thyroiditis and Grave's disease, contact
dermatitis, psoriasis, graft rejection, graft versus host
disease and sarcoidosis. Examples of TH2 or TH2-like related

disorders include atopic conditions, such as asthma and
allergy, including allergic rhinitis, gastrointestinal
allergies, including food allergies, eosinophilia,
conjunctivitis, glomerular nephritis, certain pathogen
5 susceptibilities such as helminthic (e.g., leishmaniasis) and
certain viral infections, including HIV, and bacterial
infections, including tuberculosis and lepromatous leprosy.
        Ischemic disorder or injury can be treated via the
methods of the invention. "Ischemic disorder or injury"
10 refers to any disorder or injury to tissues or organs which
results from local deficiency of the blood supply and/or of
said tissue or organs. Such deficiencies of the blood supply
and/or hypoxia are generally produced by a restriction or
obstruction of the blood supply to said tissue or organs.
15 Ischemic disorders or injuries which may be treated by the
methods of the present invention include, but are by no means
limited to, ischemic renal disease or injury, or myocardial
ischemia such as angina pectoris. Ischemic disorders or
injuries which may be treated by the methods of the present
20 invention also include damage or injury to tissue or organs
due to an infarction, such as damage to the heart, brain
(such as in a stroke), spleen, kidney, intestine, lung, and
testes. The methods of the invention may also be used to
regulate the extent or degree of ischemic injury in other
25 tissues, such as tumor tissues including, but not limited to,
tumors of the uterus and ovaries. The ischemic disorders or
injuries which may be treated by the methods of the present
invention still further include ischemic injury or damage to
transplanted organs which occurs during transplant.
30   It is further contemplated that the methods and
compositions described herein can be utilized in the
prognostic and diagnostic evaluation of disorders involving
other immune cells, including CD8+ CTLs, exhibiting TH-like
cell subpopulation gene expression patterns and/or activity.
35 It is still further contemplated that the methods and
compositions described herein can be utilized in the
amelioration of symptoms stemming from disorders involving

such immune cells, especially such CD8˙ CTLs, which exhibit
TH-like cell subpopulation gene expression patterns and/or
activity.

The invention further provides methods for the
5   identification of compounds which modulate the expression of
genes or the activity, e.g. level, of gene products involved
in TH cell subpopulation-related disorders and processes
relevant to the differentiation, maintenance and/or effector
function of the subpopulations. For example, presented
10 herein are methods for identifying compounds which affect the
level of 103 gene expression and/or gene product.

In addition, the present invention provides methods for
identifying compounds which bind to gene products of the
differentially expressed sequences identified herein. For
15 example, such methods include, but are not limited to,
methods for identifying compounds which bind to a 103 gene
product.

Still further, the present invention provides methods
for the treatment of TH cell subpopulation-related disorders
20 which can, for example, involve the administration of such
modulatory compounds to individuals exhibiting TH cell
subpopulation-related disorder symptoms or tendencies.
Additionally, treatment can result in the stimulation or
depletion of one or more of the TH cell subpopulations.

25   "Stimulation", as used herein, can refer to an effective
increase in the number of cells belonging to a TH cell
subpopulation, via, for example, the proliferation of such TH
cell subpopulation cells. The term can also refer to an
increase in the activity of cells belonging to a TH cell
30 subpopulation, as would be evidenced, for example, by a per
cell increase in the expression of the TH cell subpopulation-
specific cytokine pattern.

"Depletion", as used herein, can refer to an effective
reduction in the number of cells belonging to a TH cell
35 subpopulation, via, for example, a reduction in the
proliferation of such TH cell subpopulation cells. The term
can also refer to a decrease in the activity of cells

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belonging to a TH cell subpopulation, as would be evidenced, for example, by a per cell decrease in the expression of the TH cell subpopulation-specific cytokine pattern.

In an alternative embodiment of the present invention, 5 the methods a compositions described herein can also be used in the treatment of ischemic disorders or injuries. For example, presented herein are methods of using the 200 gene, its gene product, and antibodies thereto to treat or regulate ischemic disorders and/or injuries. In particular, the genes 10 or gene products of the invention may be administered to an individual so as to ameliorate the symptoms of the ischemic disorder or injury. Further, compounds, such as specific antibodies, including monoclonal antibodies, which bind specifically to the genes or gene products of the present 15 invention and modulate their expression or activity, may also be administered to an individual suffering from an ischemic disorder or injury.

The invention is based, in part on systematic search strategies involving paradigms which utilize TH0, TH1, TH2, 20 TH1-like and TH2-like cells, in systems which mimic the activity of the immune system or immune disorders, coupled with sensitive and high-throughput gene expression assays, to identify genes differentially expressed within and/or among TH cell subpopulations. In contrast to approaches that 25 merely evaluate the expression of a single known gene product presumed to play a role in some immune cell-related process or disorder, the search strategies and assays used herein permit the identification of all genes, whether known or novel, which are differentially expressed within and among TH 30 cell subpopulations, as well as making possible the characterization of their temporal regulation and function in the TH cell response and/or in TH cell mediated disorders. This comprehensive approach and evaluation permits the discovery of novel genes and gene products, as well as the 35 identification of a constellation of genes and gene products (whether novel or known) involved in novel pathways (e.g., modulation pathways) that play a major role in the TH-cell

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mediated immune responses and TH cell subpopulation-related
disorders. Thus, the present invention makes possible the
identification and characterization of targets useful for
prognosis, diagnosis, monitoring, rational drug design,
5 and/or therapeutic intervention of immune system disorders.
     The Examples described in Sections 6 through 8, below,
demonstrate the successful use of the search strategies of
the invention to identify genes which are differentially
expressed among and/or within TH cell subpopulations.
10 Section 9 describes the successful cloning of a human homolog
of one of the identified genes (the 200 gene).
     The 102 and 103 genes represent genes which, while
previously known, are shown here to be differentially
expressed among TH cell subpopulations. Specifically, the
15 102 gene corresponds to the Granzyme A, or Hanukah factor,
gene, which encodes a trypsin-like serine protease. While
this gene had previously been reported to be expressed in
natural killer cells and a fraction of CD4⁺ cells, the
results described herein reveal, for the first time, that the
20 gene is differentially expressed within the TH2 cell
subpopulation. Specifically, the 102 gene is expressed at a
level many-fold higher in the TH2 cell subpopulation than in
the TH1 cell subpopulation.
     The 103 gene corresponds to a gene known as the T1, ST-2
25 or Fit-1 gene, which encodes, possibly via alternative
splicing, both transmembrane and soluble gene products. The
gene 103 products belong to the immunoglobulin superfamily,
and bear a high resemblance to the interleukin-1 (IL-1)
receptor. The results presented herein demonstrate, for the
30 first time, that this gene is expressed, in vivo, in a
tightly controlled TH2-specific fashion. Thus, given its
status as both a TH2 cell subpopulation-specific marker and a
cell surface protein, the gene 103 products can be utilized
in a variety of methods to diagnose and/or modulate immune
35 system disorders, in particular TH2 cell subpopulation-
related disorders. Further, results, including results
obtained in vivo in an animal model for asthma, a TH2-like

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disorder, are presented herein which indicate that the 103
gene product provides a critical signal to TH2 effector
cells.

In addition to these known genes, the systematic search
5 strategies described herein were used to identify several
novel genes which are differentially expressed within and/or
among TH cell subpopulations. Specifically, these include
the 10, 54, 57, 105, 106, 161 and 200 genes.

The 54, 105, 106 and murine 200 genes are each shown to
10 be differentially expressed within the TH1 cell
subpopulation. Specifically, these genes are expressed at
levels many-fold higher in TH1 cell subpopulations than in
TH2 cell subpopulations.

The novel 54 gene product is a 371 amino acid cysteine
15 protease, as evidenced by the presence of three thiol
protease domains at approximately amino acid residue 145 to
156 (CYS domain), approximately amino acid residue 287 to 297
(HIS domain) and approximately amino acid residue 321 to 340
(ASN domain) of the 54 gene product amino acid sequence.
20   The 10 and 57 genes represent TH inducible gene
sequences. That is, the expression of such genes in
unstimulated TH cells is either undetectable or barely
detectable, but is significantly upregulated in both
stimulated TH1 and stimulated TH2 cells. Thus, the 10 and 57
25 genes and/or their gene products can represent new targets
for therapeutic treatment as part of a non-TH cell
subpopulation dependent intervention program.

The 10 gene product is a 338 amino acid receptor
molecule which is a particularly suitable target for such a
30 program in that the 10 gene product belongs to a class of
proteins having a seven transmembrane domain sequence motif,
which tend to represent G protein-coupled receptor molecules.
The 10 gene product structure, therefore, indicates that it
may be involved in signal transduction events which may be
35 important to T cell responses in general, and further
indicates that modulation of 10 gene product may effectively
ameliorate a wide range of T cell-related disorders.

Specifically, because the 10 gene product is a
transmembrane product, its activity, via either a physical
change in the number of 10 gene-expressing cells or by a
change in the functional level of 10 gene product activity,
5 can be particularly amenable to modulation. For example,
natural ligands, derivatives of natural ligands and
antibodies which bind to the 10 gene product can be utilized
to reduce the number of induced T cells present by either
physically separating such cells away from other cells in a
10 population, or, alternatively, by targeting the specific
destruction of the induced T cells or inhibiting the
proliferation of such T cells.

Additionally, compounds such as 10 gene sequences or
gene products such as, for example, soluble 10 gene products,
15 can be utilized to reduce the level of induced T cell
activity, and, ultimately, bring about the amelioration of a
wide range of T cell-related disorders. For example, in the
case of soluble gene 10 gene products, the compounds can
compete with the endogenous (i.e., natural) ligand for the 10
20 gene product, leading to a modulation of induced T cell
activity. Soluble proteins or peptides, such as peptides
comprising one or more of the extracellular domains, or
portions and/or analogs thereof, of the 10 gene product,
including, for example, soluble fusion proteins such as Ig-
25 tailed fusion proteins, can be particularly useful for this
purpose. Additionally, antibodies directed against one or
more of the extracellular portions of the 10 gene product may
either reduce 10 gene product function by, for example,
blocking ligand binding. Additionally, antibodies directed
30 against the 10 gene product can, in certain instances, serve
to increase the level of 10 gene product activity.

The receptor nature of the 10 gene product makes
possible useful methods for the identification of compounds
which modulate the receptor's functional activity and which
35 can act as therapeutic agents in the amelioration of a wide
range of T cell-related disorders. For example, functional
assays which measure intracellular calcium release levels may

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be utilized to identify compounds which act as either
agonists or antagonists of 10 gene product activity. Such
assays may, additionally, be utilized to identify the natural
10 gene product ligand. Still further, any of these
5 modulatory compounds can be utilized as therapeutic agents
for the amelioration of a wide range of T cell-related
disorders.

Finally, the 161 gene is shown to be an additional new
and potentially interesting target for a therapeutic method
10 aimed at the amelioration of immune disorder related
symptoms. In fact, it is possible that 161 gene expression
may be indicative of the presence of yet another TH cell
subpopulation, in addition to TH1, TH2 and TH0 cell
subpopulations.
15   The identification of TH cell subpopulation specific
markers can be utilized in the treatment of a number of
immune disorders, especially TH cell subpopulation-related
disorders. For example, markers for the TH2 subpopulation
can be used to ameliorate conditions involving an
20 inappropriate IgE immune response, including but not limited
to the symptoms which accompany atopic conditions such as
allergy and/or asthma. IgE-type antibodies are produced by
stimulated B cells which require, at least in part, IL-4
produced by the TH2 cell subpopulation. Therefore, a
25 treatment which reduces the effective concentration of
secreted IL-4, e.g., by reducing the activity or number of
TH2 cells, will bring about a reduction in the level of
circulating IgE, leading, in turn, to the amelioration or
elimination of atopic conditions. Any of the TH2-specific
30 gene products described herein can, therefore, be used as a
target to reduce or deplete the number and/or activity of TH2
cell subpopulation cells for the treatment of such
conditions.

The 103 gene can be particularly suitable for this
35 purpose since one of its gene products is a membrane-bound
TH2 cell subpopulation molecule. Accordingly, natural
ligands, derivatives of natural ligands and antibodies which

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bind to this 103 gene product, can be utilized to reduce the
number of TH2 cells present by either physically separating
such cells away from other cells in a population, or,
alternatively, by targeting the specific destruction of TH2
5 cells or inhibiting the proliferation of such TH2 cells.
Additionally, compounds such as 103 gene sequences or gene
products can be utilized to reduce the level of TH2 cell
activity, cause a reduction in IL-4 production, and,
ultimately, bring about the amelioration of IgE and/or TH2-
10 related disorders. For example, the compounds can compete
with the endogenous (i.e., natural) ligand for the 103 gene
product. The resulting reduction in the amount of ligand-
bound 103 gene transmembrane protein will modulate TH2
cellular activity. Soluble proteins or peptides, such as
15 peptides comprising the extracellular domain, the secreted
form, or portions and/or analogs thereof, of the 103 gene
product, including, for example, soluble fusion proteins such
as Ig-tailed fusion proteins, can be particularly useful for
this purpose. In certain instances, antibodies directed
20 against the 103 gene product, such as directed against the
extracellular domain of the 103 gene product, can be utilized
for this purpose.

        The identification of TH cell subpopulation specific
markers can additionally be utilized in the treatment of a
25 TH1 cell subpopulation-related disorders. For example,
markers for the TH1 cell subpopulation can be used to
ameliorate conditions involving an inappropriate cell-
mediated immune response, including, but not limited to
chronic inflammatory and autoimmune disorders. Further,
30 transgenic animals overexpressing or misexpressing such gene
sequences and/or transgenic "knockout" animals exhibiting
little or no expression of such sequences can be utilized as
animal models for TH cell subpopulation-related disorders.
The Example presented in Section 11, below, describes the
35 production of 200 and 103 transgenic animals.

        TH1 cell subpopulation specific gene sequences and/or
gene products such as the 54 (which encodes a 371 amino acid

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cysteine protease gene product), 105, 106 and 200 (the murine homolog of which encodes a 280 amino acid transmembrane gene product, the human homolog of which encodes a 301 amino acid transmembrane gene product, both of which are members of the
5 Ig superfamily) genes can, therefore, be suitable for ameliorating such TH1 cell subpopulation-related disorders.

The 200 gene product can be particularly suitable for such a purpose in that it is not only TH1 cell subpopulation-restricted, but the Ig superfamily 200 gene product is,
10 additionally, membrane-bound. Therefore, natural ligands, derivatives of natural ligands and antibodies which bind to the 200 gene product can be utilized to reduce the number of TH1 cells present by either physically separating such cells away from other cells in a population, or, alternatively, by
15 targeting the specific destruction of TH1 cells or inhibiting the proliferation of such TH1 cells. Additionally, compounds such as 200 gene sequences or gene products such as soluble 200 gene products, can be utilized to reduce the level of TH2 cell activity, thus bringing about the amelioration of TH1
20 cell subpopulation-related disorders. For example, the compounds can compete with the endogenous (i.e., natural) ligand for the 200 gene product. The resulting reduction in the amount of ligand-bound 200 gene transmembrane protein will modulate TH2 cellular activity. Soluble proteins or
25 peptides, such as peptides comprising the extracellular domain, or portions (such as, for example, the Ig portion) and/or analogs thereof, of the 200 gene product, including, for example, soluble fusion proteins such as Ig-tailed fusion proteins, can be particularly useful for this purpose. The
30 Example presented in Section 10, below, describes the construction and expression of 200 gene product and 103 gene product Ig fusion constructs and proteins.

Further, the Example presented in Section 12, below, describes successful use of antibodies directed against the
35 103 gene product as well as 103/Ig fusion proteins to ameliorate symptoms of asthma in an accepted animal model for the TH2-related disorder. Thus, the results indicate that

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the 103 gene product provides a critical signal to TH2 cells
and can successfully be used as a target for selective
modulation of TH immune responses (e.g., for selective
suppression of TH2 immune responses and/or selective
5 enhancement of TH1 immune responses).

    The invention is also based, in part, on the discovery
that among the genes and gene products described herein are
ones also involved in processes related to tissue repair and
remodeling after injury, particularly after ischemic injury.
10 In particular, the example presented in Section 13, below,
demonstrates the successful use of antibodies which bind to
the extracellular domain of the 200 gene product to inhibit
repair of ischemic kidney injury. Thus, the invention also
makes possible the treatment of ischemic disorders and
15 injuries. The invention is also based, in part, on the
discovery that the 103 gene is expressed in mast cells, as
demonstrated in the Example presented in Section 14, below.

3.1. DEFINITIONS

20   The term "TH cell subpopulation", as used herein, refers
to a population of TH cells exhibiting a gene expression
pattern (e.g., a discrete pattern of cytokines and/or
receptor or other cell surface molecules) and activity which
are distinct from the expression pattern and activity of
25 other TH cells. Such TH cell subpopulations can include, but
are not limited to, TH0, TH1 and TH2 subpopulations, which
will, for clarity and example, and not by way of limitation,
be frequently used herein as representative TH cell
subpopulations.

30   The term "TH-like cell subpopulation" (e.g., "TH1-like"
or "TH2-like"), as used herein is intended to refer not only
to a population of CD4$^+$ TH cells having the properties
described, above, for a TH cell subpopulation, but also
refers to CD4$^-$ cells, including CD8$^+$ CTLs, which exhibit TH-
35 like cytokine expression patterns.

"Differential expression", as used herein, refers to
both quantitative as well as qualitative differences in the
genes' temporal and/or cellular expression patterns.

"Target gene", as used herein, refers to a
5   differentially expressed gene involved in immune disorders
and/or in the differentiation, maintenance and/or effector
function of TH cell subpopulations, such that modulation of
the level of target gene expression or of target gene product
presence and/or activity can, for example, act to result in
10 the specific depletion or repression, or, alternatively, the
stimulation or augmentation of one or more TH cell
subpopulation, bringing about, in turn, the amelioration of
symptoms of immune disorders, e.g., TH cell subpopulation-
related disorders. A target gene can also exhibit
15 fingerprint and/or pathway gene characteristics.

"Fingerprint gene," as used herein, refers to a
differentially expressed gene whose mRNA expression pattern,
protein level and/or activity can be utilized as part of a
prognostic or diagnostic in the evaluation of immune
20 disorders, e.g., TH cell subpopulation-related disorders, or
which, alternatively, can be used in methods for identifying
compounds useful for the treatment of such disorders, by, for
example, evaluating the effect of the compound on the
fingerprint gene expression normally displayed in connection
25 with the disease. A fingerprint gene can also exhibit target
and/or pathway gene characteristics.

"Fingerprint pattern," as used herein, refers to the
pattern generated when the mRNA expression pattern, protein
level and/or activity of a series (which can range from two
30 up to all the fingerprint genes which exist for a given
state) of fingerprint genes is determined. A fingerprint
pattern can be a part of the same methods described, above,
for the expression of a single fingerprint gene.

"Pathway genes", as used herein, refers to a gene whose
35 product exhibits an ability to interact with gene products
involved in immune disorders, e.g., TH cell subpopulation-
related disorders and/or to interact with gene products which

are involved in the differentiation and effector function of
TH cell subpopulations. Pathway genes can also exhibit
target gene and/or fingerprint gene characteristics.

        "Negative modulation", as used herein, refers to a
5 reduction in the level and/or activity of target gene product
relative to the level and/or activity of the target gene
product in the absence of the modulatory treatment.
Alternatively, the term, as used herein, refers to a
reduction in the number and/or activity of cells belonging to
10 the TH cell subpopulation relative to the number and/or
activity of the TH cell subpopulation in the absence of the
modulatory treatment.

        "Positive modulation", as used herein, refers to an
increase in the level and/or activity of target gene product
15 relative to the level and/or activity of the gene product in
the absence of the modulatory treatment. Alternatively, the
term, as used herein, refers to an increase in the number
and/or activity of cells belonging to the TH cell
subpopulation, relative to the number and/or activity of the
20 TH cell subpopulation in the absence of the modulatory
treatment.

        "Ischemic disorder or injury", as used herein, refers to
any disorder or injury to tissues or organs which results
from local deficiency of the blood supply and/or hypoxia to
25 said tissue or organs. Such deficiency of the blood supply
and/or hypoxia is generally produced by a restriction or
obstruction of the blood supply to said tissue or organs.

        The term "mast-cell related processes or disorders", as
used herein, includes, but is not limited to, atherosclerosis
30 and myocardial ischemia/reperfusion.

4.   DESCRIPTION OF THE FIGURES

        FIG. 1. Differential display analysis of RNA from
murine TH cell subsets. Splenic T cells derived from T cell
35 receptor transgenic mice were differentiated in vitro to
become polarized populations of TH1 or TH2 subtypes. Lane 1:
TH2 population 24 hours after tertiary stimulation; lane 2:

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TH1 population 24 hours after tertiary stimulation; lane 3:
TH2 population 1 week after secondary stimulation; lane 4:
TH1 population 1 week after secondary stimulation; lane 5:
TA3 cell line, which was used as antigen presenting cell
5 (APC) for in vitro stimulation. (This sample was used as a
negative control.) Each set of lanes consists of duplicates
(a and b), in which cDNAs were independently generated from
the same source of RNA. Arrow points to differentially
expressed sequence, which is referred to herein as band 102.
10   Further, the gene corresponding to band 102 is referred
to herein as the 102 gene. All lanes are products of a
polymerase chain reaction (PCR) in which $T_{11}GG$ was used as the
3' oligonucleotide and a random 10mer oligonucleotide (Oligo
#4, OP-D kit, Operon, Inc.) was used as the 5'
15 oligonucleotide.

     FIG. 2. Nucleotide sequence of clone 102.1 of band 102
(SEQ. ID NO: 1). The gene corresponding to band 102 is
referred to herein as the 102 gene.
20

     FIG. 3. Northern blot analysis of confirming
differential regulation of the 102 gene within primary
TH1/TH2 cultures and murine tissues. RNA was harvested from
T cell lines derived from a T cell receptor transgenic strain
25 stimulated in vitro. Lane 1, TH2, 40 hours after second
stimulation; lane 2, TH1, 40 hours after second stimulation;
lane 3, TH2 population 24 hours after tertiary stimulation;
lane 4, TH1, 24 hours after tertiary stimulation; lane 5,
murine thymus; lane 6, murine spleen. Five micrograms of
30 total RNA was used per lane. The cloned band 102 sequence
was used as a probe.

     FIG. 4A. Nucleotide sequence clone 103.1 of band 103
(SEQ ID NO:2). The gene corresponding to band 103 is
35 referred to herein as gene 103.

FIG. 4B. 103 gene products. This diagram illustrates
the relationship between the sequence encoded by band 103,
103 gene (also known as ST-2, T1 and Fit-1) products and the
IL-1 receptor polypeptide structure. The extracellular,
5 transmembrane and cytoplasmic domains of the proteins are
noted, along with the amino acid residues marking the
boundaries of these domains. (Adapted from Yanagisawa et
al., 1993, FEBS Lett. 318:83-87.)

10   FIG. 4C. The nucleotide sequence encoding the secreted
form of murine 103 gene product is depicted (SEQ ID NO:49;
Accession No. E07714).

FIG. 4D. The amino acid sequence of the secreted form
15 of murine 103 is depicted (SEQ ID NO:47; Accession No.
P14719).

FIG. 4E. The nucleotide sequence encoding the
transmembrane murine 103 gene product is depicted (SEQ ID
20 NO:38; Accession No. E08652).

FIG. 4F. The amino acid sequence of the transmembrane
receptor of murine 103 is depicted (SEQ ID NO:39; Accession
No. S29498). The extracellular domain of the full length,
25 transmembrane product extends from amino acid residue 1 to
342 of SEQ ID NO: 38 (SEQ ID NO:41), the transmembrane domain
of the full length, transmembrane product extends from amino
acids 343 to 366 of SEQ ID NO: 38 (SEQ ID NO:48) the
intracellular domain of the full length, transmembrane
30 product extends from amino acid residues 367 to 567 of SEQ ID
NO: 38 (SEQ ID NO:43).

FIG. 4G. The nucleotide sequence encoding the secreted
product of the human 103 gene is depicted (SEQ ID NO:44;
35 Accession No. NM_003856).

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FIG. 4H. The amino acid sequence of the secreted
product of the human 103 gene is depicted (SEQ ID NO:45;
Accession No. NM_003856).

5   FIG. 5. Quantitative RT-PCR analysis of 103 gene
expression in polarized populations of murine TH cells. RNA
samples were harvested from cultured T cell populations 24
hours after tertiary stimulation with antigen. cDNA samples
were PCR amplified and the products of those reactions were
10 electrophoresed on a 1% agarose gel and visualized by
ethidium bromide staining. 103 gene expression is shown in
the upper panel. γ-actin data, bottom panel, was included as
a control for differences in sample quality. The numbers
above each lane represent the dilution factors of each cDNA.
15 The same cDNA samples were used for both the 103 gene and the
γ-actin amplifications.

FIG. 6. Northern blot analysis of 103 gene expression
in representative murine TH cell lines (TH2: CDC25, D10.G4,
20 DAX; TH1: AE7.A, Dorris, D1.1). Clones were either
unstimulated (-) or stimulated (+) for 6 hours with plate-
bound anti-CD3 antibody. Ten micrograms of total RNA were
loaded per lane. The positions of 18s and 28s ribosomal RNA
are shown as reference markers.
25

FIG. 7. Northern blot analysis of 103 gene expression
in T cell clones and murine tissues. Lane 1: DAX cells, no
stimulation; lane 2, AE7 cells, stimulation; lane 3, AE7
cells, no stimulation; lane 4, D10.G4 cells, stimulation;
30 lane 5, D10.G4 cells, no stimulation; lane 6, brain; lane 7,
heart; lane 8, lung; lane 9, spleen; lane 10, liver. Clones
were stimulated with plate-bound anti-CD3 antibody for 6
hours. 7.5 and 10 micrograms total RNA was used for each
cell line and each tissue, respectively. a, b, and c arrows
35 refer to RNA encoding full length (a) and truncated (b,c)
forms of the 103 gene. The positions of 18s and 28s
ribosomal RNA markers are shown.

FIG. 8. RNAse protection analysis of 103 gene mRNA,
illustrating regulation of 103 gene expression in murine TH
cell clones. Lanes 2-6: β-actin protection; lanes 9-13:
103 gene protection; lanes 1 and 8: markers; lanes 2 and 9:
5 unstimulated TH1 clones; lanes 3 and 10: stimulated TH1
clones; lanes 4 and 11: unstimulated TH2 clones; lanes 5 and
12: stimulated TH2 clones; lanes 6 and 13: fully RNAse A
digested unprotected probe; lanes 7 and 14: probe alone, in
absence of added RNAse.

10   Expected fragment sizes:
        β-actin protected probe: 250 nucleotides;
        β-actin full length probe: 330 nucleotides;
        103 gene long form fragment: 257 nucleotides;
        103 gene short form fragment: 173 nucleotides;
15   103 gene full length probe: 329 nucleotides.

FIG. 9. The full length 10 gene nucleotide sequence
(SEQ ID NO: 3) is shown on the top line, while the derived
amino acid sequence of the 10 gene product (SEQ ID NO: 9) is
20 shown on the bottom line. The underlined portion of the
nucleotide sequence corresponds to the band 10 nucleotide
sequence. The data shown in FIG. 10A-C was obtained through
the use of the portion of the 10 gene product which is
encoded by the band 10 nucleotide sequence.
25
        FIG. 10A-C. 10 gene hydrophilicity data, indicating
that the 10 gene-derived amino acid sequence predicts the
presence of a seven transmembrane domain structural motif.
10A) platelet activating factor receptor hydrophilicity plot
30 illustrating the protein's seven transmembrane domain
structural motif; 10B) 10 gene hydrophilicity plot
illustrating a portion of the protein's putative seven
transmembrane domain structural motif; 10C) platelet
activating factor receptor hydrophilicity plot illustrating
35 part of the protein's seven transmembrane structural motif.

FIG. 11. Chromosomal mapping of locus containing the 10 gene sequence. A map of a portion of mouse chromosome 12 is shown. Numbers to left of chromosome are in centiMorgans; D12NDS11, D12MIT4, and D12MIT8 represent mouse microsatellite
5 markers; TH10 represents 10 gene.

FIG. 12. Nucleotide sequence of clone 7 of band 57 (SEQ ID NO:4). The gene corresponding to band 57 is referred to herein as the 57 gene.
10

FIG. 13. Consensus nucleotide sequence of band 105 (SEQ ID NO:5). "N" signifies "any nucleotide". The gene corresponding to band 105 is referred to herein as the 105 gene.
15

FIG. 14. Nucleotide sequence obtained from clone H of band 106 (SEQ ID NO:6). "N" signifies "any nucleotide". The gene corresponding to band 106 is referred to herein as the 106 gene.
20

FIG. 15. Nucleotide sequence of clone G of band 161 (SEQ ID NO:7). The gene corresponding to band 161 is referred to herein as the 161 gene.

25   FIG. 16. Multiple sequence alignment of 161 clone G with amino acid sequences identified in a BLAST search. Asterisks signify positions that are identical; dots indicate conserved positions.

30   FIG. 17. Nucleotide and amino acid sequence of the full length murine 200 gene. Bottom line: murine 200 gene nucleotide sequence (SEQ ID NO:8); top line: murine 200 gene product derived amino acid sequence (SEQ ID NO: 10).

35   FIG. 18. Northern blot analysis of murine 200 gene expression in representative murine TH cell lines (TH2: CDC25, D10.G4, DAX; TH1: AE7.A, Dorris, D1.1). Clones were

either unstimulated (-) or stimulated (+) for 6 hours with
plate-bound anti-CD3 antibody. The positions of RNA markers,
in kilobases, are shown for reference. The arrow marks the
position of 200 gene mRNA.

5

        FIG. 19. Northern blot analysis of 54 gene expression
within TH1 (D1.1, Dorris, AE7) cell lines and TH2 (D10.G4,
DAX, CDC25) cell lines, either stimulated (+) or unstimulated
(-) with anti-CD3 antibodies. 15 micrograms of total RNA
10 were loaded per lane. Cells were stimulated between 6 and 7
hours with anti-CD3 antibodies, as described, below, in
Section 8.1. The Northern blots were hybridized with a probe
made from the entire band 54 nucleotide sequence.

15   FIG. 20. Northern blot analysis of gene 54 time course
study. RNA from TH1 cell line AE7 cells was isolated, either
unstimulated or stimulated for varying periods of time, as
indicated. Second, RNA from two TH2 cell lines (DAX, CDC25)
was isolated from either unstimulated cells or from cells
20 which had been stimulated for two hours with anti-CD3
antibodies. 15 micrograms total RNA were loaded.per lane. A
band 54 DNA probe was used for hybridization.

        FIG. 21. Northern blot analysis of 54 gene expression
25 in various tissues. 15 micrograms of total RNA were loaded
per lane. A band 54· DNA probe was used for hybridization.

        FIG. 22. Nucleotide and amino acid sequence of the full
length 54 gene. Bottom line: 54 gene nucleotide sequence
30 (SEQ ID NO:11). Top line: 54 gene derived amino acid
sequence (SEQ ID NO:12).

        FIG. 23. The 54 gene product bears a high level of
homology to the cysteine protease class of proteins. The 54
35 gene product amino acid is depicted with its predicted pre-
pro sequence and mature cysteine protease polypeptide
sequence identified. The individual boxed amino acid

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residues represent residues thought to lie within the
cysteine protease active site and the stretch of amino acid
residues which are boxed represent a region with homology to
a stretch of amino acid residues normally seen within the
5 preproenzyme portion of cysteine protease molecules. The
circled amino acid residues within this stretch represent
conserved amino acids. The arrow indicates the putative
post-translational cleavage site.

10   FIG. 24. Nucleotide and amino acid sequence of the full
length human 200 gene. Bottom line: human 200 gene
nucleotide sequence (SEQ ID NO: 23); top line: human 200
gene product derived amino acid sequence (SEQ ID NO:24).

15   FIG. 25. Flow cytometry data demonstrates that the 3E10
mAb recognizes and binds to representative clones of the TH2
cell subpopulation (D10.G4; DAX), but not clones of the TH1
subtype (AE7; Dorris). The graphs in this figure present the
results of the flow cytometry analyses by depicting the
20 number of cells exhibiting a given level of fluorescence.
Staining above background levels represents antigen-specific
binding and, therefore, the presence of cell surface 103 gene
product. The further to the right the peaks are shifted, the
greater the staining intensity, and therefore antibody
25 binding, exhibited by a cell population.

Fig. 26. Analysis of the cytokine profile in mouse BAL.
The data presented in this figure reveals high levels of IL-
4, IL-5, IL-6, IL-10 and IL-13 in TH2 recipient OVA
30 challenged mice (closed bars). There was no detectable TH2
cytokines in the BAL fluid of mice that received TH2 cells
and were not exposed to ovalbumin. Pretreatment with 3E10
mAb resulted in a dramatic reduction in IL-4, IL-5, IL-6 and
IL-13, but had no effect on IL-10 levels in the BAL (open
35 bars). OVA challenge of TH1 recipient mice resulted in high
levels of IFN-γ in the BAL fluid (closed bars) that was not

inhibited by 3E10 mAb (open bars). Data are shown as the
mean ± sem of 5-6 animals.

5   Fig. 27A-27B.   Anti-103 gene product mAb inhibits TH2
mediated allergic lung inflammation. FIG. 27A: Analysis of
the number of eosinophils in the BAL; FIG. 27B: analysis of
the number of lymphocytes in the BAL.   The number of OVA-
specific TH2 cells in dispersed lung tissue as described
(Cohn, L. et al., 1997, J. Exp. Med. 186:1737-1747).
10 Lymphocytes were stained with biotinylated clonotypic TCR mAb
KJ126 (Cohn, L. et al., 1997 J. Exp. Med. 186:1737-1747)
followed by strepavidin-FITC and CD4-PE (Pharmingen, San
Diego).

15   FIG. 28.   Inhibition of airway hyperresponsiveness by
anti-103 gene product mAb.   OVA exposure in TH2 recipient
mice resulted in airway hyperresponsiveness (closed squares)
compared to mice exposed to PBS (closed circles).
Pretreatment with 103 gene product mAb inhibited OVA induced
20 BHR by 80% (open diamonds). The results are shown as the mean
Penh ± sem of n = 5-6 and is representative of 2 separate
experiments.

      Fig. 29A-29B.   Administration of 3E10 mAb or the 103/Ig
25 fusion results in significant decrease in hallmark symptoms
of asthma.   FIG. 29A: Animals were treated with the anti-103
3E10 antibody (listed in the figure as "3E10 mAB").   As a
negative control, a set of animals was treated with a non-
specific rat Ig antibody preparation.   FIG. 29B: Animals
30 were treated with 103/Ig fusion protein (listed in the figure
as "Ig Fus. Prot.") as a negative control, a control set of
animals were treated with a non-specific human IgG antibody
preparation.

35   Fig. 30.   Crosslinking of 103 gene product augments IL-4
and IL-5 cytokine secretion.   TH2 effector cells were
activated with plate-bound CD3 (3 μg/ml, 2C11) and CD28

                            - 29 -

(37.51, 4 μg/ml, Pharmingen San Diego) and 3E10 (20 μg/ml)
for 48 hrs. IL-4 and IL-5 levels were measured in the
supernatant by ELISA. 3E10 mAb stimulation alone failed to
induce TH2 cell activation but augmented both anti-CD3 and
5 anti-CD3 + CD28 induced cytokine production. Soluble 3E10
failed to have any effect on CD3/CD28 mediated cytokine
production. These data suggest that activation of 103 gene
product provides a stimulatory signal to TH2 cells. There
was no effect of the mAb on TH2 cell proliferation as
10 revealed by ^{3}H-thymidine incorporation. 3E10 mAb did not
modify IFN-γ secretion from TH1 effector cells stimulated
under the same conditions.

        Fig. 31. Renal histology at 72 hours reperfusion; Fig.
15 31A shows a section of untreated mouse kidney tissue; Fig.
31B shows a section of mouse kidney tissue treated with 200
gene antibody 24 hours prior to, and at 24 hour intervals
after the induction of ischemic kidney injury.

20   Fig. 32. Histological scoring of gene 200 blockage in
treated (+a200) and untreated (+RtIg) mouse kidney tissue
during renal ischemia/reperfusion injury (RI), and in non
ischemic controls (S).

25   5. DETAILED DESCRIPTION OF THE INVENTION
        Methods and compositions for the treatment and diagnosis
of immune disorders, especially TH cell subpopulation-related
disorders, including, but not limited to, atopic conditions,
such as asthma and allergy, including allergic rhinitis,
30 psoriasis, the effects of pathogen infection, chronic
inflammatory diseases, organ-specific autoimmunity, graft
rejection and graft versus host disease, are described. The
methods and compositions described herein can also be used to
treat ischemic disorders and injuries, including but not
35 limited to, ischemic renal disease and injury, myocardial
ischemia such as angina pectoris, as well as ischemic injury
to other tissues, including the brain (as in a stroke),

                              - 30 -

spleen, intestine, lung, and testes. Further, the methods
and compositions described herein can also be used to
regulate ischemic injury to other types of tissue, such as
tumor tissue including, but not limited to tumors of the
5 ovary and uterus. The invention is based, in part, on the
evaluation of the expression and role of all genes that are
differentially expressed within and/or among TH cell
subpopulations in paradigms that are physiologically relevant
to TH-mediated immune response and/or TH-subpopulation
10 related disorders. This permits the definition of disease
pathways that are useful both diagnostically and
therapeutically. The invention is also based in part on the
discovery that the genes and gene products of the invention
are also involved in processes related to tissue repair and
15 remodeling after injury, particularly after ischemic injury.
Thus, the genes and gene products of the invention can also
be used to successfully treat such injuries and related
disorders.

        Genes, termed "target genes" and/or "fingerprint genes",
20 which are differentially expressed within and among TH cells
and TH cell subpopulations in normal and/or disease states,
and/or during the differentiation into such mature
subpopulations are described in Section 5.4. Additionally,
genes, termed "pathway genes", whose gene products exhibit an
25 ability to interact with gene products involved in TH cell
subpopulation-related disorders and/or with gene products
which are involved in the differentiation and effector
function of the subpopulations are described in Section 5.4.
Pathway genes can additionally have fingerprint and/or target
30 gene characteristics. Methods for the identification of such
fingerprint, target, and pathway genes are also described in
Sections 5.1 and 5.2.

        Further, the gene products of such fingerprint, target,
and pathway genes are described in Section 5.5, antibodies to
35 such gene products are described in Section 5.6, as are cell-
and animal-based models of TH cell subpopulation

differentiation and TH cell subpopulation-related disorders
to which such gene products can contribute in Section 5.7.

Methods for prognostic and diagnostic evaluation of
various TH cell subpopulation-related disorders, for the
5 identification of subjects exhibiting a predisposition to
such disorders, and for monitoring the efficacy of compounds
used in clinical trials are described in Section 5.12.

Methods for the identification of compounds which
modulate the expression of genes or the activity of gene
10 products involved in (a) TH cell subpopulation-related
disorders, (b) the differentiation and effector function of
TH cell subpopulations, and (c) processes related to tissue
repair and remodeling after ischemic injury are described in
Section 5.8. Methods for the treatment of immune disorders
15 and ischemic disorders and injuries are described in Section
5.10.

5.1.   IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES

Described herein are methods for the identification of
20 differentially expressed genes which are involved in immune
disorders, e.g., TH cell subpopulation-related disorders,
and/or which are involved in the differentiation, maintenance
and effector function of the subpopulations. There exist a
number of levels at which the differential expression of such
25 genes can be exhibited. For example, differential expression
can occur in undifferentiated TH cells versus differentiated
or differentiating TH cells (although not necessarily within
one TH cell subpopulation versus another), in naive TH cells
versus memory TH cells, within one TH cell subpopulation
30 versus another (e.g., TH1 versus TH2 subpopulations), in
mature, stimulated cells versus mature, unstimulated cells of
a given TH cell subpopulation or in TH cell subpopulation-
related disorder states relative to their expression in
normal, or non-TH cell subpopulation-related disorder states.
35 Such differentially expressed genes can represent target
and/or fingerprint genes.

Methods for the identification of such differentially expressed genes are described, below, in Section 5.1.1. Methods for the further characterization of such differentially expressed genes, and for their categorization

5 as target and/or fingerprint genes, are presented, below, in Section 5.3.

"Differential expression" as used herein refers to both quantitative as well as qualitative differences in the genes' temporal and/or cell type expression patterns. Thus, a

10 differentially expressed gene can qualitatively have its expression activated or completely inactivated in, for example, normal versus TH cell subpopulation-related disorder states, in one TH cell subpopulation versus another (e.g., TH1 versus TH2), in antigen stimulated versus unstimulated

15 sets of TH cells, or in undifferentiated versus differentiated or differentiating TH cells. Such a qualitatively regulated gene will exhibit an expression pattern within a state or cell type which is detectable by standard techniques in one such state or cell type, but is

20 not detectable in both.

Alternatively, a differentially expressed gene can exhibit an expression level which differs, i.e., is quantitatively increased or decreased, in normal versus TH cell subpopulation-related disorder states, in antigen

25 stimulated versus unstimulated sets of TH cells, in one TH cell subpopulation versus another, or in undifferentiated versus differentiated or differentiating TH cells. Because differentiation is a multistage event, genes which are differentially expressed can also be identified at any such

30 intermediate differentiative stage.

The degree to which expression differs need only be large enough to be visualized via standard characterization techniques, such as, for example, the differential display technique described below. Other such standard

35 characterization techniques by which expression differences can be visualized include, but are not limited to,

quantitative RT (reverse transcriptase) PCR and Northern
analyses and RNase protection techniques.

Differentially expressed genes can be further described
as target genes and/or fingerprint genes. "Fingerprint
5 gene," as used herein, refers to a differentially expressed
gene whose expression pattern can be utilized as part of a
prognostic or diagnostic TH cell subpopulation-related
disorder evaluation, or which, alternatively, can be used in
methods for identifying compounds useful for the treatment of
10 TH cell subpopulation-related disorders. A fingerprint gene
can also have the characteristics of a target gene or a
pathway gene (see below, in Section 5.2).

"Fingerprint pattern," as used herein, refers to the
pattern generated when the expression pattern of a series
15 (which can range from two up to all the fingerprint genes
which exist for a given state) of fingerprint genes is
determined. A fingerprint pattern can also be used in
methods for identifying compounds useful in the treatment of
immune disorders, e.g., by evaluating the effect of the
20 compound on the fingerprint pattern normally displayed in
connection with the disease.

"Target gene", as used herein, refers to a
differentially expressed gene involved in TH cell
subpopulation-related disorders and/or in differentiation,
25 maintenance and/or effector function of the subpopulations in
a manner by which modulation of the level of target gene
expression or of target gene product activity can act to
ameliorate symptoms of TH cell subpopulation-related
disorders. For example, such modulation can result either
30 the depletion or stimulation of one or more TH cell
subpopulation, which, in turn, brings about the amelioration
of immune disorder, e.g., TH cell subpopulation disorder,
symptoms.

"Stimulation", as used herein, can refer to an effective
35 increase in the number of cells belonging to a T cell
population, such as a TH cell subpopulation, via, for
example, the proliferation of such TH cell subpopulation

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cells. The term can also refer to an increase in the
activity of cells belonging to a TH cell subpopulation, as
would by evidenced, for example, by a per cell increase in
the expression of the TH cell subpopulation-specific cytokine
5 pattern.

"Depletion", as used herein, can refer to an effective
reduction in the number of cells belonging to a T cell
population, such as a TH cell subpopulation, via, for
example, a reduction in the proliferation of such TH cell
10 subpopulation cells. The term can also refer to a decrease
in the activity of cells belonging to a TH cell
subpopulation, as would be evidenced, for example, by a per
cell decrease in the expression of the TH cell subpopulation-
specific cytokine pattern.

15   TH cell subpopulation-related disorders include, for
example, atopic conditions, such as asthma and allergy,
including allergic rhinitis, the effects of pathogen,
including viral, infection, chronic inflammatory diseases,
psoriasis, glomerular nephritis, organ-specific autoimmunity,
20 graft rejection and graft versus host disease. A target gene
can also have the characteristics of a fingerprint gene
and/or a pathway gene (as described, below, in Section 5.2).

5.1.1.   METHODS FOR THE IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES

25   A variety of methods can be utilized for the
identification of genes which are involved in immune disorder
states, e.g., TH cell subpopulation-related disorder states,
and/or which are involved in differentiation, maintenance
30 and/or effector function of the subpopulations. Described in
Section 5.1.1.1 are experimental paradigms which can be
utilized for the generation of subjects and samples which can
be used for the identification of such genes. Material
generated in paradigm categories can be characterized for the
35 presence of differentially expressed gene sequences as
discussed, below, in Section 5.1.1.2.

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5.1.1.1. PARADIGMS FOR THE IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES

Paradigms which represent models of normal and abnormal immune responses are described herein. These paradigms can be utilized for the identification of genes which are differentially expressed within and among TH cell subpopulations, including but not limited to TH1 and TH2 subpopulations. Such genes can be involved in, for example, TH cell subpopulation differentiation, maintenance, and/or effector function, and in TH cell subpopulation-related disorders. For example, TH cells can be induced to differentiate into either TH1 or TH2 states, can be stimulated with, for example, a foreign antigen, and can be collected at various points during the procedure for analysis of differential gene expression.

In one embodiment of such a paradigm, referred to herein as the "transgenic T cell paradigm", transgenic animals, preferably mice, are utilized which have been engineered to express a particular T cell receptor, such that the predominant T cell population of the immune system of such a transgenic animal recognizes only one antigen. Such a system is preferred in that it provides a source for a large population of identical T cells whose naivete can be assured, and whose response to the single antigen it recognizes is also assured. T helper cells isolated from such a transgenic animal are induced, in vitro, to differentiate into TH cell subpopulations such as TH1, TH2, or TH0 cell subpopulations. In a specific embodiment, one T helper cell group (the TH1 group) is exposed to IL-12, a cytokine known to induce differentiation into the TH1 state, a second T helper cell group (the TH2 group) is exposed to IL-4, a cytokine known to induce differentiation into the TH2 state, and a third group is allowed, by a lack of cytokine-mediated induction, to enter a TH-undirected state.

A second paradigm, referred to herein as a "T cell line paradigm", can be utilized which uses mature TH cell clones, such as TH1 and TH2 and TH1-like and TH2-like cell lines,

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preferably human cell lines. Such TH cell lines can include, but are not limited to the following well known murine cell lines: Doris, AE7, D10.G4, DAX, D1.1 and CDC25. Such T cell lines can be derived from normal individuals as well as

5 individuals exhibiting TH cell subpopulation-related disorders, such as, for example, chronic inflammatory diseases and disorders, such as Crohn's disease, reactive arthritis, including Lyme disease, insulin-dependent diabetes, organ-specific autoimmunity, including multiple

10 sclerosis, Hashimoto's thyroiditis and Grave's disease, contact dermatitis, psoriasis, graft rejection, graft versus host disease, sarcoidosis, atopic conditions, such as asthma and allergy, including allergic rhinitis, gastrointestinal allergies, including food allergies, eosinophilia,

15 conjunctivitis, glomerular nephritis, certain pathogen susceptibilities such as helminthic (e.g., leishmaniasis) and certain viral infections, including HIV, and bacterial infections, including tuberculosis and lepromatous leprosy.

The TH cell clones can be stimulated in a variety of

20 ways. Such stimulation methods include, but are not limited to, pharmacological methods, such as exposure to phorbol esters, calcium ionophores, or lectins (e.g., Concanavalin A), by treatment with antibodies directed against T-cell receptor epitopes (e.g., anti-CD3 antibodies) or exposure, in

25 the presence of an appropriate antigen presenting cell (APC), to an antigen that the particular TH cells are known to recognize. Following such primary stimulation, the cells can be maintained in culture without stimulation and, for example, in the presence of IL-2, utilizing standard

30 techniques well known to those of skill in the art. The cells can then be exposed to one or more additional cycles of stimulation and maintenance.

A third paradigm, referred to herein as an "in vivo paradigm", can also be utilized to discover differentially

35 expressed gene sequences. In vivo stimulation of animal models forms the basis for this paradigm. The in vivo nature of the stimulation can prove to be especially predictive of

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the analogous responses in living patients. Stimulation can
be accomplished via a variety of methods. For example,
animals, such as transgenic animals described earlier in this
Section, can be injected with appropriate antigen and
5   appropriate cytokine to drive the desired TH cell
differentiation. Draining lymph nodes can then be harvested
at various time points after stimulation. Lymph nodes from,
for example, TH1-directed animals can be compared to those of
TH2-directed animals.
10   A wide range of animal models, representing both models
of normal immune differentiation and function as well as
those representing immune disorders can be utilized for this
in vivo paradigm. For example, any of the animal models,
both recombinant and non-recombinant, described, below, in
15 Section 5.7.1, can be used.
        Cell samples can be collected during any point of such a
procedure. For example, cells can be obtained following any
stimulation period and/or any maintenance period.
Additionally, cells can be collected during various points
20 during the TH cell differentiation process. RNA collected
from such samples can be compared and analyzed according to,
for example, methods described, below, in Section 5.1.1.2.
For example, RNA from TH0, TH1 and TH2 groups isolated at a
given time point can then be analyzed and compared.
25 Additionally, RNA from stimulated and non-stimulated cells
within a given TH cell group can also be compared and
analyzed. Further, RNA collected from undifferentiated TH
cells can be compared to RNA collected from cells at various
stages during the differentiative process which ultimately
30 yields TH cell subpopulations.

            5.1.1.2. ANALYSIS OF PARADIGM MATERIAL
        In order to identify differentially expressed genes,
RNA, either total or mRNA, can be isolated from the TH cells
35 utilized in paradigms such as those described in Section
    5.1.1.1. Any RNA isolation technique which does not select
    against the isolation of mRNA can be utilized for the

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purification of such RNA samples. See, for example, Ausubel, F.M. et al., eds., 1987-1993, <u>Current Protocols in Molecular Biology</u>, John Wiley & Sons, Inc. New York, which is incorporated herein by reference in its entirety.

5   Additionally, large numbers of cell samples can readily be processed using techniques well known to those of skill in the art, such as, for example, the single-step RNA isolation process of Chomczynski, P. (1989, U.S. Patent No. 4,843,155), which is incorporated herein by reference in its entirety.

10   Transcripts within the collected RNA samples which represent RNA produced by differentially expressed genes can be identified by utilizing a variety of methods which are well known to those of skill in the art. For example, differential screening (Tedder, T.F. <u>et al.</u>, 1988, Proc.

15   Natl. Acad. Sci. USA <u>85</u>:208-212), subtractive hybridization (Hedrick, S.M. <u>et al.</u>, 1984, Nature <u>308</u>:149-153; Lee, S.W. <u>et al.</u>, 1984, Proc. Natl. Acad. Sci. USA <u>88</u>:2825), and, preferably, differential display (Liang, P. and Pardee, A.B., 1992, Science <u>257</u>:967-971; U.S. Patent No. 5,262,311, which

20   are incorporated herein by reference in their entirety), can be utilized to identify nucleic acid sequences derived from genes that are differentially expressed.

        Differential screening involves the duplicate screening of a cDNA library in which one copy of the library is

25   screened with a total cell cDNA probe corresponding to the mRNA population of one cell type while a duplicate copy of the cDNA library is screened with a total cDNA probe corresponding to the mRNA population of a second cell type. For example, one cDNA probe can correspond to a total cell

30   cDNA probe of a cell type or tissue derived from a control subject, while the second cDNA probe can correspond to a total cell cDNA probe of the same cell type or tissue derived from an experimental subject. Those clones which hybridize to one probe but not to the other potentially represent

35   clones derived from genes differentially expressed in the cell type of interest in control versus experimental subjects.

Subtractive hybridization techniques generally involve the isolation of mRNA taken from two different sources, the hybridization of the mRNA or single-stranded cDNA reverse-transcribed from the isolated mRNA, and the removal of all

5 hybridized, and therefore double-stranded, sequences. The remaining non-hybridized, single-stranded cDNAs, potentially represent clones derived from genes that are differentially expressed among the two mRNA sources. Such single-stranded cDNAs are then used as the starting material for the

10 construction of a library comprising clones derived from differentially expressed genes.

The differential display technique is a procedure, utilizing the well-known polymerase chain reaction (PCR; the experimental embodiment set forth in Mullis, K.B., 1987, U.S.

15 Patent No. 4,683,202), which allows for the identification of sequences derived from genes which are differentially expressed. First, isolated RNA is reverse-transcribed into single-stranded cDNA, utilizing standard techniques which are well known to those of skill in the art. Primers for the

20 reverse transcriptase reaction can include, but are not limited to, oligo dT-containing primers, preferably of the 3' primer type of oligonucleotide described below.

Next, this technique uses pairs of PCR primers, as described below, which allow for the amplification of clones

25 representing a reproducible subset of the RNA transcripts present within any given cell. Utilizing different pairs of primers allows each of the primed mRNA transcripts present in a cell to be amplified. Among such amplified transcripts can be identified those which have been produced from

30 differentially expressed genes.

The 3' oligonucleotide primer of the primer pairs can contain an oligo dT stretch of 10-13, preferably 11, dT nucleotides at its 5' end, which hybridizes to the poly(A) tail of mRNA or to the complement of a cDNA reverse

35 transcribed from an mRNA poly(A) tail. In order to increase the specificity of the 3' primer, the primer can contain one or more, preferably two, additional nucleotides at its 3'

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end. Because, statistically, only a subset of the mRNA
derived sequences present in the sample of interest will
hybridize to such primers, the additional nucleotides allow
the primers to amplify only a subset of the mRNA derived
5 sequences present in the sample of interest. This is
preferred in that it allows more accurate and complete
visualization and characterization of each of the bands
representing amplified sequences.

The 5' primer can contain a nucleotide sequence
10 expected, statistically, to have the ability to hybridize to
cDNA sequences derived from the cells or tissues of interest.
The nucleotide sequence can be an arbitrary one, and the
length of the 5' oligonucleotide primer can range from about
9 to about 15 nucleotides, with about 13 nucleotides being
15 preferred.

Arbitrary primer sequences cause the lengths of the
amplified partial cDNAs produced to be variable, thus
allowing different clones to be separated by using standard
denaturing sequencing gel electrophoresis.
20   PCR reaction conditions should be chosen which optimize
amplified product yield and specificity, and, additionally,
produce amplified products of lengths which can be resolved
utilizing standard gel electrophoresis techniques. Such
reaction conditions are well known to those of skill in the
25 art, and important reaction parameters include, for example,
length and nucleotide sequence of oligonucleotide primers as
discussed above, and annealing and elongation step
temperatures and reaction times.

The pattern of clones resulting from the reverse
30 transcription and amplification of the mRNA of two different
cell types is displayed via sequencing gel electrophoresis
and compared. Differentially expressed genes are indicated
by differences in the two banding patterns.

Once potentially differentially expressed gene sequences
35 have been identified via bulk techniques such as, for
example, those described above, the differential expression
of such putatively differentially expressed genes should be

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corroborated. Corroboration can be accomplished via, for
example, such well known techniques as Northern analysis,
quantitative RT/PCR, or RNAse protection.

   Upon corroboration, the differentially expressed genes
5 can be further characterized, and can be identified as target
and/or fingerprint genes, as discussed, below, in Section
5.3.

   The amplified sequences of differentially expressed
genes obtained through, for example, differential display can
10 be used to isolate full length clones of the corresponding
gene. The full length coding portion of the gene can readily
be isolated, without undue experimentation, by molecular
biological techniques well known in the art. For example,
the isolated differentially expressed amplified fragment can
15 be labeled and used to screen a cDNA library. Alternatively,
the labeled fragment can be used to screen a genomic library.

   PCR technology can also be utilized to isolate full
length cDNA sequences. As described, above, in this Section,
the isolated, amplified gene fragments obtained through
20 differential display have 5' terminal ends at some random
point within the gene and usually have 3' terminal ends at a
position corresponding to the 3' end of the transcribed
portion of the gene. Once nucleotide sequence information
from an amplified fragment is obtained, the remainder of the
25 gene (i.e., the 5' end of the gene, when utilizing
differential display) can be obtained using, for example, RT-
PCR.

   In one embodiment of such a procedure for the
identification and cloning of full length gene sequences, RNA
30 can be isolated, following standard procedures, from an
appropriate tissue or cellular source. A reverse
transcription reaction can then be performed on the RNA using
an oligonucleotide primer complimentary to the mRNA that
corresponds to the amplified fragment, for the priming of
35 first strand synthesis. Because the primer is anti-parallel
to the mRNA, extension will proceed toward the 5' end of the
mRNA. The resulting RNA/DNA hybrid can then be "tailed" with

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guanines using a standard terminal transferase reaction, the
hybrid can be digested with RNAase H, and second strand
synthesis can then be primed with a poly-C primer. Using the
two primers, the 5' portion of the gene is amplified using
5 PCR. Sequences obtained can then be isolated and recombined
with previously isolated sequences to generate a full-length
cDNA of the differentially expressed genes of the invention.
For a review of cloning strategies and recombinant DNA
techniques, see e.g., Sambrook et al., 1989, Molecular
10 Cloning, A Laboratory Manual, (Volumes 1-3) Cold Spring
Harbor Press, N.Y.; and Ausubel et al., 1989, Current
Protocols in Molecular Biology, Green Publishing Associates
and Wiley Interscience, N.Y.

15   5.2. METHODS FOR THE IDENTIFICATION OF PATHWAY GENES
        Methods are described herein for the identification of
pathway genes. "Pathway gene", as used herein, refers to a
gene whose gene product exhibits the ability to interact with
gene products involved in TH cell subpopulation-related
20 disorders and/or to interact with gene products which are
involved in differentiation, maintenance and/or effector
function of TH cell subpopulations. A pathway gene can be
differentially expressed and, therefore, can have the
characteristics of a target and/or fingerprint gene, as
25 described, above, in Section 5.1.
        Any method suitable for detecting protein-protein
interactions can be employed for identifying pathway gene
products by identifying interactions between gene products
and gene products known to be involved in TH cell
30 subpopulation-related disorders and/or involved in
differentiation, maintenance, and/or effector function of the
subpopulations. Such known gene products can be cellular or
extracellular proteins. Those gene products which interact
with such known gene products represent pathway gene products
35 and the genes which encode them represent pathway genes.
        Among the traditional methods which can be employed are
co-immunoprecipitation, crosslinking and co-purification

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through gradients or chromatographic columns. Utilizing
procedures such as these allows for the identification of
pathway gene products. Once identified, a pathway gene
product can be used, in conjunction with standard techniques,
5 to identify its corresponding pathway gene. For example, at
least a portion of the amino acid sequence of the pathway
gene product can be ascertained using techniques well known
to those of skill in the art, such as via the Edman
degradation technique (see, e.g., Creighton, 1983, "Proteins:
10 Structures and Molecular Principles", W.H. Freeman & Co.,
N.Y., pp.34-49). The amino acid sequence obtained can be
used as a guide for the generation of oligonucleotide
mixtures that can be used to screen for pathway gene
sequences. Screening can be accomplished, for example, by
15 standard hybridization or PCR techniques. Techniques for the
generation of oligonucleotide mixtures and for screening are
well-known. (See, e.g., Ausubel, supra., and PCR Protocols:
A Guide to Methods and Applications, 1990, Innis, M. et al.,
eds. Academic Press, Inc., New York).

20   Additionally, methods can be employed which result in
the simultaneous identification of pathway genes which encode
proteins interacting with a protein involved in TH cell
subpopulation-related disorder states and/or differentiation,
maintenance, and/or effector function of the subpopulations.
25 These methods include, for example, probing expression
libraries with labeled protein known or suggested to be
involved in the disorders and/or the differentiation,
maintenance, and/or effector function of the subpopulations,
using this protein in a manner similar to the well known
30 technique of antibody probing of λgt11 libraries.

One method which detects protein interactions in vivo,
the two-hybrid system, is described in detail for
illustration purposes only and not by way of limitation. One
version of this system has been described (Chien et al.,
35 1991, Proc. Natl. Acad. Sci. USA, 88:9578-9582) and is
commercially available from Clontech (Palo Alto, CA).

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Briefly, utilizing such a system, plasmids are
constructed that encode two hybrid proteins: one consists of
the DNA-binding domain of a transcription activator protein
fused to a known protein, in this case, a protein known to be
5 involved in TH cell subpopulation differentiation or effector
function, or in TH cell subpopulation-related disorders, and
the other consists of the activator protein's activation
domain fused to an unknown protein that is encoded by a cDNA
which has been recombined into this plasmid as part of a cDNA
10 library. The plasmids are transformed into a strain of the
yeast <u>Saccharomyces cerevisiae</u> that contains a reporter gene
(e.g., <u>lacZ</u>) whose regulatory region contains the
transcription activator's binding sites. Either hybrid
protein alone cannot activate transcription of the reporter
15 gene, the DNA-binding domain hybrid cannot because it does
not provide activation function, and the activation domain
hybrid cannot because it cannot localize to the activator's
binding sites. Interaction of the two hybrid proteins
reconstitutes the functional activator protein and results in
20 expression of the reporter gene, which is detected by an
assay for the reporter gene product.

The two-hybrid system or related methodology can be used
to screen activation domain libraries for proteins that
interact with a known "bait" gene product. By way of
25 example, and not by way of limitation, gene products known to
be involved in TH cell subpopulation-related disorders and/or
differentiation, maintenance, and/or effector function of the
subpopulations can be used as the bait gene products. Total
genomic or cDNA sequences are fused to the DNA encoding an
30 activation domain. This library and a plasmid encoding a
hybrid of the bait gene product fused to the DNA-binding
domain are cotransformed into a yeast reporter strain, and
the resulting transformants are screened for those that
express the reporter gene. For example, and not by way of
35 limitation, the bait gene can be cloned into a vector such
that it is translationally fused to the DNA encoding the DNA-
binding domain of the GAL4 protein. These colonies are

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purified and the library plasmids responsible for reporter
gene expression are isolated. DNA sequencing is then used to
identify the proteins encoded by the library plasmids.

A cDNA library of the cell line from which proteins that
5 interact with bait gene product are to be detected can be
made using methods routinely practiced in the art. According
to the particular system described herein, for example, the
cDNA fragments can be inserted into a vector such that they
are translationally fused to the activation domain of GAL4.
10 This library can be co-transformed along with the bait gene-
GAL4 fusion plasmid into a yeast strain which contains a lacZ
gene driven by a promoter which contains GAL4 activation
sequence. A cDNA encoded protein, fused to GAL4 activation
domain, that interacts with bait gene product will
15 reconstitute an active GAL4 protein and thereby drive
expression of the lacZ gene. Colonies which express lacZ can
be detected by their blue color in the presence of X-gal.
The cDNA can then be purified from these strains, and used to
produce and isolate the bait gene-interacting protein using
20 techniques routinely practiced in the art.

Once a pathway gene has been identified and isolated, it
can be further characterized as, for example, discussed
below, in Section 5.3.

25   5.3.   CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED AND
                        PATHWAY GENES
Differentially expressed genes, such as those identified
via the methods discussed, above, in Section 5.1, and pathway
genes, such as those identified via the methods discussed,
30 above, in Section 5.2, above, as well as genes identified by
alternative means, can be further characterized by utilizing,
for example, methods such as those discussed herein. Such
genes will be referred to herein as "identified genes".

Analyses such as those described herein yield
35 information regarding the biological function of the
identified genes. An assessment of the biological function
of the differentially expressed genes, in addition, will

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allow for their designation as target and/or fingerprint
genes.

Specifically, any of the differentially expressed genes
whose further characterization indicates that a modulation of
5 the gene's expression or a modulation of the gene product's
activity can ameliorate any of the TH cell subpopulation-
related disorders of interest will be designated "target
genes", as defined, above, in Section 5.1. Such target genes
and target gene products, along with those discussed below,
10 will constitute the focus of the compound discovery
strategies discussed, below, in Section 5.8. Further, such
target genes, target gene products and/or modulating
compounds can be used as part of the TH cell subpopulation-
disorder treatment methods described, below, in Section 5.9.
15 Such methods can include, for example, methods whereby the TH
cell subpopulation of interest is selectively depleted or
repressed, or, alternatively, stimulated or augmented.

Any of the differentially expressed genes whose further
characterization indicates that such modulations can not
20 positively affect TH cell subpopulation-related disorders of
interest, but whose expression pattern contributes to a gene
expression "fingerprint" pattern correlative of, for example,
a TH1/TH2-related disorder state, will be designated a
"fingerprint gene". "Fingerprint patterns" will be more
25 fully discussed, below, in Section 5.12.1. It should be
noted that each of the target genes can also function as
fingerprint genes, as well as can all or a portion of the
pathway genes.

It should further be noted that the pathway genes can
30 also be characterized according to techniques such as those
described herein. Those pathway genes which yield
information indicating that modulation of the gene's
expression or a modulation of the gene product's activity can
ameliorate any a TH cell subpopulation-related disorder will
35 also be designated "target genes". Such target genes and
target gene products, along with those discussed above, will
constitute the focus of the compound discovery strategies

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discussed, below, in Section 5.8 and can be used as part of
the treatment methods described in Section 5.9, below.

In instances wherein a pathway gene's characterization
indicates that modulation of gene expression or gene product
5 activity can not positively affect TH cell subpopulation-
related disorders of interest, but whose expression is
differentially expressed and contributes to a gene expression
fingerprint pattern correlative of, for example, a TH1/TH2-
related disorder state, such pathway genes can additionally
10 be designated as fingerprint genes.

A variety of techniques can be utilized to further
characterize the identified genes. First, the nucleotide
sequence of the identified genes, which can be obtained by
utilizing standard techniques well known to those of skill in
15 the art, can, for example, be used to reveal homologies to
one or more known sequence motifs which can yield information
regarding the biological function of the identified gene
product.

Second, an analysis of the tissue and/or cell type
20 distribution of the mRNA produced by the identified genes can
be conducted, utilizing standard techniques well known to
those of skill in the art. Such techniques can include, for
example, Northern, RNAse protection, and RT-PCR analyses.
Such analyses provide information as to, for example, whether
25 the identified genes are expressed in cell types expected to
contribute to the specific TH cell subpopulation-related
disorders of interest. Such analyses can also provide
quantitative information regarding steady state mRNA
regulation, yielding data concerning which of the identified
30 genes exhibits a high level of regulation in cell types which
can be expected to contribute to the TH cell subpopulation-
related disorders of interest. Additionally, standard in
situ hybridization techniques can be utilized to provide
information regarding which cells within a given tissue or
35 population of cells express the identified gene. Such an
analysis can provide information regarding the biological
function of an identified gene relative to a given TH cell

subpopulation-related disorder in instances wherein only a
subset of the cells within a tissue or a population of cells
is thought to be relevant to the disorder.

Third, the sequences of the identified genes can be
5 used, utilizing standard techniques, to place the genes onto
genetic maps, e.g., mouse (Copeland, N.G. and Jenkins, N.A.,
1991, Trends in Genetics 7:113-118) and human genetic maps
(Cohen, D., et al., 1993, Nature 366:698-701). Such mapping
information can yield information regarding the genes'
10 importance to human disease by, for example, identifying
genes which map within genetic regions to which known genetic
TH cell subpopulation-related disorders map. Such regions
include, for example, the mouse Scl-1 locus, which is
suspected to be involved in Leishmaniasis, or the human
15 5q31.1 chromosomal region which contains one or more loci
thought to regulate IgE production in a nonantigen-specific
fashion, and can, therefore, be involved in allergy, a TH2-
like-related disorder (Marsh, D. et al., 1994, Science
264:1152-1156).
20   Fourth, the biological function of the identified genes
can be more directly assessed by utilizing relevant in vivo
and in vitro systems. In vivo systems can include, but are
not limited to, animal systems which naturally exhibit the
symptoms of immune disorders, or ones which have been
25 engineered to exhibit such symptoms. Further, such systems
can include systems for the further characterization of the
cell type differentiation and effector function, and can
include, but are not limited to transgenic animal systems
such as those described, above, in Section 5.1.1.1, and
30 Section 5.7.1, below. In vitro systems can include, but are
not limited to, cell-based systems comprising, for example,
TH1 or TH2 cell types. The TH subpopulation cells can be
wild type cells, or can be non-wild type cells containing
modifications known or suspected of contributing to the TH
35 cell subpopulation-related disorder of interest. Such
systems are discussed in detail, below, in Section 5.7.2.

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In further characterizing the biological function of the identified genes, the expression of these genes can be modulated within the in vivo and/or in vitro systems, i.e., either overexpressed or underexpressed in, for example,
5 transgenic animals and/or cell lines, and its subsequent effect on the system can then be assayed. Alternatively, the activity of the product of the identified gene can be modulated by either increasing or decreasing the level of activity in the in vivo and/or in vitro system of interest,
10 and its subsequent effect then assayed.

The information obtained through such characterizations can suggest relevant methods for the treatment or control of immune disorders, such as TH cell subpopulation-related disorders, involving the gene of interest. For example,
15 relevant treatment can include not only a modulation of gene expression and/or gene product activity, but can also include a selective depletion or stimulation of the TH cell subpopulation of interest. Characterization procedures such as those described herein can indicate where such modulation
20 should be positive or negative. As used herein, "positive modulation" refers to an increase in gene expression or activity of the gene or gene product of interest, or to a stimulation of a TH cell subpopulation, relative to that observed in the absence of the modulatory treatment.
25 "Negative modulation", as used herein, refers to a decrease in gene expression or activity, or a depletion of a TH cell subpopulation, relative to that observed in the absence of the modulatory treatment. "Stimulation" and "depletion" are as defined, above, in Section 3. Methods of treatment are
30 discussed, below, in Section 5.9.

5.4. DIFFERENTIALLY EXPRESSED AND PATHWAY GENES

Differentially expressed genes such as those identified in Section 5.1.1, above, and pathway genes, such as those
35 identified in Section 5.2, above, are described herein.

The differentially expressed and pathway genes of the invention are listed below, in Table 1. Differentially

expressed gene sequences are shown in FIGS. 2, 4A, 9 and 12-
15, 17, 22 and 24. The nucleotide sequences identified via
differential display analysis are referred to herein as band
10, 54, 57, 102, 103, 105, 106, 161 and 200. The genes
5   corresponding to these sequences are referred to herein as
the 10, 54, 57, 102, 103, 106, 161 and 200 genes,
respectively. Table 1 lists differentially expressed genes
identified through, for example, the paradigms discussed,
above, in Section 5.1.1.1, and below, in the Examples
10 presented in Sections 6-8.

    Table 1 summarizes information regarding the further
characterization of such genes. Table 2 lists E. coli
clones, deposited with the Agricultural Research Service
Culture Collection (NRRL) or the American Type Culture
15 Collection (ATCC), which contain sequences found within the
genes of Table 1.

    In Table 1, the column headed "Diff. Exp." details the
differential expression characteristic by which the sequence
has been identified. Under this column, "TH Inducible",
20 refers to those cases where differential expression arises
upon exposure of the cell type of interest to an agent
capable of bringing about TH cell stimulation or activation.
These sequences, therefore, are differentially expressed in
undifferentiated, partially or fully differentiated TH cells,
25 and the genes corresponding to these sequences are expressed
in both TH1 and TH2 cell subpopulations.

    "TH1", under this column, refers to a sequence
corresponding to a gene expressed preferentially in mature,
fully differentiated TH1 cells relative to TH2 cells. "TH2",
30 under this column, refers to a sequence corresponding to a
gene preferentially expressed in mature, fully differentiated
TH2 cell subpopulations relative to TH1 cell subpopulations.
Preferential expression can be qualitative or quantitative,
as described, above, in Section 5.1.

35   Tissue expression patterns are also summarized in Table
1. The column headed "Tissue/Cell Dist." lists tissues
and/or cell types in which expression of the gene has been

tested and whether expression of the gene within a given
tissue or cell type has been observed. Specifically, "+"
indicates detectable mRNA from the gene of interest, while "-
" refers to no detectable mRNA from the gene of interest.
5 Unless otherwise noted, "+" and "-" refer to all samples of a
given tissue or cell type tested. "Detectable", as used
herein, is as described, above, in Section 5.1.

Additionally, the physical locus to which the gene maps
on the human and/or mouse chromosome map is indicated in the
10 column headed "Locus". Further, in instances wherein the
genes correspond to genes known to be found in nucleic acid
databases, references (i.e., citations and/or gene names) to
such known genes are listed in the column headed "Ref.".

The genes listed in Table 1 can be obtained using
15 cloning methods well known to those of skill in the art, and
include but are not limited to the use of appropriate probes
to detect the genes within an appropriate cDNA or gDNA
(genomic DNA) library. (See, for example, Sambrook et al.,
1989, Molecular Cloning: A Laboratory Manual, Cold Spring
20 Harbor Laboratories, which is incorporated herein by
reference in its entirety.) Probes for the sequences
reported herein can be obtained directly from the isolated
clones deposited with the NRRL, as indicated in Table 2,
below. Alternatively, oligonucleotide probes for the genes
25 can be synthesized based on the DNA sequences disclosed
herein in FIGS. 2, 4A, 9, 12-15, 17, 22 and 24. With respect
to the previously reported genes, synthetic oligonucleotides
can be synthesized or produced based on the sequences
provided for the previously known genes described in the
30 following references: granzyme A, Hanukah factor: Masson,
D. et al., 1986, FEBS Lett. 208:84-88; Masson, D. et al.,
1986, EMBO J. 5:1595-1600; Gershenfeld, H.K. and Weissman,
I.L., 1986, Science 232:854-858; ST-2, Tl, Fit-1: Klemenz,
R. et al., 1989, Proc. Natl. Acad. Sci. USA 86:5708-5712;
35 Tominaga, S., 1989, FEBS Lett. 258:301-301; Werenskiold, A.K.
et al., 1989, Mol. Cell. Biol. 9:5207-5214; Tominaga, S. et
al., 1992, Biochem. Biophys. Acta. 1171:215-218; Werenskiold,

A.K., 1992, Eur. J. Biochem. <u>204</u>:1041-1047; Yanagisawa, K. et
al., 1993, FEBS Lett. <u>318</u>:83-87; Bergers, G. et al., 1994,
EMBO J. <u>13</u>:1176-1188; and Kumar, S., 1997, Biochem. Biophys.
Res. Comm. <u>235</u>:474-478.

5   The probes can be used to screen cDNA libraries prepared
from an appropriate cell or cell line in which the gene is
transcribed. Appropriate cell lines can include, for
example, Dorris, AE7, D10.G4, DAX, D1.1 and CDC25 cell lines.
In addition, purified primary naive T cells derived from
10 either transgenic or non-transgenic strains can be used.
Alternatively, the genes described herein can be cloned from
a cDNA library constructed from, for example, NIH 3T3 cell
lines stably transfected with the Ha-ras(EJ) gene, 5C10
cells, and peripheral blood lymphocytes.
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TABLE 1
DIFFERENTIALLY EXPRESSED AND PATHWAY GENES

Gene	Diff. Exp.	Tissue/ Cell Dist.	Locus	Ref
102	TH2	TH2 Specific		ref1
103	TH2	(+) TH2 (-) Lymph Node; Spleen; Thymus; Brain; Lung; Bone Marrow; Heart; Spleen.		ref2
10	TH Inducible	(+) Spleen; TH1; TH2. (-) Liver; Brain; Thymus; Bone Marrow; Heart; Lymph Node.	See FIG. 11	
57	TH Inducible	(+) TH1; TH2; Spleen		
105	TH1	(+) TH1; Spleen		
106	TH1	(+) TH1; Thymus; Spleen		
161	Subset Specific[3]	(+) Spleen (-) Thymus		
200	TH1	(+) TH1		
54	TH1	(+) TH1; spleen; testis; uterus (-)brain; heart; kidney; liver; muscle		

[1]   Masson, D. et al., 1986, FEBS Lett. 208:84-88;
Masson, D. et al., 1986, EMBO J. 5:1595-1600; Gershenfeld,
H.K. and Weissman, I.L., 1986, Science 232:854-858.

[2]   Klemenz, R. et al., 1989, Proc. Natl. Acad. Sci.
USA 86:5708-5712; Tominaga, S., 1989, FEBS Lett. 258:301-301;
Werenskiold, A.K. et al., 1989, Mol. Cell. Biol.9:5207-5214;
Tominaga, S. et al., 1992, Biochem. Biophys. Acta. 1171:215-
218; Werenskiold, A.K., 1992, Eur. J. Biochem. 204:10-1047;
Yanagisawa, K. et al., 1993, FEBS Lett. 318:83-87; Bergers,
5 G. et al., 1994, EMBO J. 13:1176-1188 and Kumar, S, 1997,
Biochem Biophys Res Commun 235:474-478.

[3] Band 161 expression has been observed in either TH1 or
TH2 cell subpopulations, but has not been found,
simultaneously, in both TH1 and TH2 cell subpopulations.

10

       Table 2, below, lists isolated E. coli clones which
contain sequences within the novel genes listed in Table 1.

TABLE 2

GENE		CLONE
10		10-C
10		10-X
57		57-E
105		105-A
106		106-H
161		161-G
200	(murine)	200-O
200	(murine)	DH10B(Zip)™ containing 200-P
200	(murine)	200-AF
200	(human)	feht 200-C
54		54-C
200	(human)	feht 200-C

       As used herein, "differentially expressed gene" (i.e.
target and fingerprint gene) or "pathway gene" refers to (a)
a gene containing: at least one of the DNA sequences and/or
fragments thereof that are disclosed herein (as shown in
35 FIGS. 2, 4A, 9, 12-15, 17, 22 and 24), or contained in the
clones listed in Table 2, as deposited with the NRRL or ATCC;
(b) any DNA sequence or fragment thereof that encodes the

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amino acid sequence encoded by: the DNA sequences disclosed
herein (as shown in FIGS. 2, 4A, 9, 12-15, 17, 22 and 24),
contained in the clones, listed in Table 2, as deposited with
the NRRL or ATCC contained within the coding region of the
5 gene to which the DNA sequences disclosed herein (as shown in
FIGS. 2, 4A, 9, 12-15, 17, 22 and 24) belong or contained in
the clones listed in Table 2, as deposited with the NRRL or
ATCC, belong; (c) any DNA sequence that hybridizes to the
complement of: the coding sequences disclosed herein (as
10 shown in FIGS. 2, 4A, 9, 12-15, 17, 22 and 24), contained in
clones listed in Table 2, as deposited with the NRRL or ATCC,
or contained within the coding region of the gene to which
the DNA sequences disclosed herein (as shown in FIGS. 2, 4A,
9, 12-15, 17, 22 and 24) belong or contained in the clones
15 listed in Table 2, as deposited with the NRRL or ATCC, under
stringent conditions, e.g., hybridization to filter-bound DNA
in 6x sodium chloride/sodium citrate (SSC) at about 45ºC
followed by one or more washes in 0.2xSSC/0.1% SDS at about
50-65ºC, or under highly stringent conditions, e.g.,
20 hybridization to filter-bound nucleic acid in 6xSSC at about
45ºC followed by one or more washes in 0.1xSSC/0.2% SDS at
about 68ºC, or under other hybridization conditions which are
apparent to those of skill in the art (see, for example,
Ausubel, F.M. et al., eds., 1989, *Current Protocols in*
25 *Molecular Biology*, Vol. I, Green Publishing Associates, Inc.
and John Wiley & Sons, Inc., New York, at pp. 6.3.1-6.3.6 and
2.10.3).

Preferably, the nucleic acid molecules that hybridize to
the complements of the DNA sequences disclosed herein encode
30 gene products, e.g., gene products that are functionally
equivalent to a gene product encoded by a gene of (a), above.

"Functionally equivalent", as utilized herein, refers to
a protein capable of exhibiting a substantially similar <u>in</u>
<u>vivo</u> activity as the endogenous differentially expressed or
35 pathway gene products encoded by the differentially expressed
or pathway gene sequences described in Section 5.4, above.
Alternatively, when utilized as part of assays such as those

described, below, in Section 5.3, "functionally equivalent"
can refer to peptides capable of interacting with other
cellular or extracellular molecules in a manner substantially
similar to the way in which the corresponding portion of the
5 endogenous differentially expressed or pathway gene product
would. Functionally equivalent gene products therefore
include naturally occurring differentially expressed or
pathway gene products present in the same or different
species. Functionally equivalent differentially expressed
10 and/or pathway gene products also include gene products that
retain at least one of the biological activities of the
differentially expressed and/or pathway gene products
described above; e.g., which are encoded by the coding
sequences disclosed herein (as shown in FIGS. 2, 4A, 9, 12-
15 15, 17, 22 and 24), contained in clones listed in Table 2, as
deposited with the NRRL or ATCC. The functionally equivalent
gene products of the pathway and/or differentially expressed
genes of the invention also include gene products which are
recognized by and bind to antibodies (polyclonal or
20 monoclonal) directed against the differentially expressed
and/or pathway gene products described above; e.g., which
are encoded by the coding sequences disclosed herein (as
shown in FIGS. 2, 4A, 9, 12-15, 17, 22 and 24), contained in
clones listed in Table 2, as deposited with the NRRL or ATCC.
25   The invention also includes degenerate variants of
sequences (a) through (d).
        The invention encompasses the following nucleotides,
host cells expressing such nucleotides and the expression
products of such nucleotides: (a) nucleotides that encode a
30 mammalian differentially expressed and/or pathway gene
product including, but not limited to a human and murine 10,
54, 57, 105, 106, 161 and 200 gene product; (b) nucleotides
that encode portions of differentially expressed and/or
pathway gene product that corresponds to its functional
35 domains, and the polypeptide products encoded by such
nucleotide sequences, and in which, in the case of receptor-
type gene products, such domains include, but are not limited

to extracellular domains (ECD), transmembrane domains (TM)
and cytoplasmic domains (CD); (c) nucleotides that encode
mutants of a differentially expressed and/or pathway gene,
product, in which all or part of one of its domains is
5 deleted or altered, and which, in the case of receptor-type
gene products, such mutants include, but are not limited to,
soluble receptors in which all or a portion of the TM is
deleted, and nonfunctional receptors in which all or a
portion of CD is deleted; and (d) nucleotides that encode
10 fusion proteins containing a differentially expressed and/or
pathway gene product or one of its domains fused to another
polypeptide.

The nucleotide sequences of the invention further
include nucleotide sequences corresponding to the nucleotide
15 sequences of (a)-(d) above wherein one or more of the exons,
or fragments thereof, have been deleted.

The nucleotide sequences of the invention still further
include nucleotide sequences that have at least 65%, 70%,
75%, 80%, 85%, 90%, 95%, 98% or more nucleotide sequence
20 identitiy to the nucleotide sequences of (a)-(d) above. The
nucleotide sequences of the invention also include nucleotide
sequences that encode polypeptides having at least 65%, 70%,
75%, 80%, 85%, 90%, 95%, 98% or higher amino acid sequence
identity to the polypeptides encoded by the nucleotide
25 sequences of (a)-(d) above.

To determine the percent identity of two amino acid
sequences or of two nucleic acid sequences, the sequences are
aligned for optimal comparison purposes (e.g., gaps can be
introduced in the sequence of a first amino acid or nucleic
30 acid sequence for optimal alignment with a second amino aor
nucleic acid sequence). The amino acid residues or
nucleotides at corresponding amino acid positions or
nucleotide positions are then compared. When a position in
the first sequence is occupied by the same amino acid residue
35 or nucleotide as the corresponding position in the second
sequence, then the molecules are identical at that position.
The percent identity between the two sequences is a function

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of the number of identical positions shared by the sequences (*i.e.*, % identity = # of identical overlapping positions/total # of positions x 100%). In one embodiment, the two sequences are the same length.

5   The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990)

10 *Proc. Natl. Acad. Sci. U.S.A.* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-0. BLAST nucleotide searches can be performed

15 with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the present invention. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score-50,

20 wordlength=3 to obtain amino acid sequences homologous to a protein molecule of the present invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-BLAST can be used to

25 perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, http://www.ncbi.nlm.nih.gov). Another

30 preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, (1988) *CABIOS* 4:11-17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When

35 utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

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The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

5   The invention also includes nucleic acid molecules, preferably DNA molecules, that hybridize to, and are therefore the complements of, the DNA sequences (a) through (d), in the preceding paragraph. Such hybridization conditions can be highly stringent or less highly stringent,
10 as described above. The nucleic acid molecules of the invention that hybridize to the above described DNA sequences include oligodeoxyoligonucleotides ("oligos") which hybridize under highly stringent or stringent conditions to the DNA sequences (a) through (d) in the preceding paragraph. In
15 general, for oligos between 14 and 70 nucleotides in length the melting temperature (Tm) is calculated using the formula: $Tm(^{\circ}C) = 81.5 + 16.6(\log[\text{monovalent cations (molar)}] + 0.41$ (% G+C) - (500/N), where N is the length of the probe. If the hybridization is carried out in a solution containing
20 formamide, the melting temperature may be calculated using the equation: $Tm(^{\circ}C) = 81.5 + 16.6(\log[\text{monovalent cations (molar)}]) + 0.41(\% \ G+C) - (0.61\% \ \text{formamide}) - (500/N)$ where N is the length of the probe. In general, hybridization is carried out at about 20-25 degrees below Tm (for DNA-DNA
25 hybrids) or about 10-15 degrees below Tm (for RNA-DNA hybrids). Other examplary highly stringent conditions may refer, e.g., to washing in 6xSSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos).

30   These nucleic acid molecules can encode or act as target gene antisense molecules, useful, for example, in target gene regulation and/or as antisense primers in amplification reactions of target, fingerprint, and/or pathway gene nucleic acid sequences. Further, such sequences can be used as part
35 of ribozyme and/or triple helix sequences, also useful for target gene regulation. Still further, such molecules can be used as components of diagnostic methods whereby the presence

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of, or predisposition to, an immune disorder, e.g., TH cell
subpopulation-related disorder, can be detected.

Fragments of the differentially expressed and pathway
genes of the invention can be at least 10 nucleotides in
5 length. In alternative embodiments, the fragments can be
about 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400,
500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or
more contiguous nucleotides in length. Alternatively, the
fragments can comprise sequences that encode at least 10, 20,
10 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450 or more
contiguous amino acid residues of the differentially express
and pathway gene products. Fragments of the differentially
expressed and pathway nucleic acid molecules of the invention
can also refer to exons or introns of the above described
15 nucleic acid molecules, as well as portions of the coding
regions of such nucleic acid molecules that encode function
domains such as extracellular domains (ECD), transmembrane
domains (TM) and cytoplasmic domains (CD).

The invention also encompasses (a) DNA vectors that
20 contain any of the foregoing coding sequences and/or their
complements (i.e., antisense); (b) DNA expression vectors
that contain any of the foregoing coding sequences
operatively associated with a regulatory element that directs
the expression of the coding sequences; and (c) genetically
25 engineered host cells that contain any of the foregoing
coding sequences operatively associated with a regulatory
element that directs the expression of the coding sequences
in the host cell. As used herein, regulatory elements
include but are not limited to inducible and non-inducible
30 promoters, enhancers, operators and other elements known to
those skilled in the art that drive and regulate expression.
Such regulatory elements include but are not limited to the
cytomegalovirus hCMV immediate early gene, the early or late
promoters of SV40 adenovirus, the lac system, the trp system,
35 the TAC system, the TRC system, the major operator and
promoter regions of phage A, the control regions of fd coat
protein, the promoter for 3-phosphoglycerate kinase, the

promoters of acid phosphatase, and the promoters of the yeast
α-mating factors. The invention includes fragments of any of
the DNA sequences disclosed herein.

In addition to the gene sequences described above,
5 homologs of these gene sequences and/or full length coding
sequences of these genes, as can be present in the same or
other species, can be identified and isolated, without undue
experimentation, by molecular biological techniques well
known in the art. Further, there can exist genes at other
10 genetic loci within the genome of the same species that
encode proteins which have extensive homology to one or more
domains of such gene products. These genes can also be
identified via similar techniques.

For example, the isolated differentially expressed gene
15 sequence can be labeled and used to screen a cDNA library
constructed from mRNA obtained from the organism of interest.
Hybridization conditions should be of a lower stringency when
the cDNA library was derived from an organism different from
the type of organism from which the labeled sequence was
20 derived. cDNA screening can also identify clones derived
from alternatively spliced transcripts in the same or
different species. Alternatively, the labeled fragment can
be used to screen a genomic library derived from the organism
of interest, again, using appropriately stringent conditions.
25 Low stringency conditions will be well known to those of
skill in the art, and will vary predictably depending on the
specific organisms from which the library and the labeled
sequences are derived. For guidance regarding such
conditions see, for example, Sambrook et al., 1989, Molecular
30 Cloning, A Laboratory Manual, Cold Springs Harbor Press,
N.Y.; and Ausubel et al., 1989, Current Protocols in
Molecular Biology, (Green Publishing Associates and Wiley
Interscience, N.Y.).

Further, a previously unknown differentially expressed
35 or pathway gene-type sequence can be isolated by performing
PCR using two degenerate oligonucleotide primer pools
designed on the basis of amino acid sequences within the gene

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of interest. The template for the reaction can be cDNA
obtained by reverse transcription of mRNA prepared from human
or non-human cell lines or tissue known or suspected to
express a differentially expressed or pathway gene allele.
5 The PCR product can be subcloned and sequenced to insure that
the amplified sequences represent the sequences of a
differentially expressed or pathway gene-like nucleic acid
sequence.

    The PCR fragment can then be used to isolate a full
10 length cDNA clone by a variety of methods. For example, the
amplified fragment can be used to screen a bacteriophage cDNA
library. Alternatively, the labeled fragment can be used to
screen a genomic library.

    PCR technology can also be utilized to isolate full
15 length cDNA sequences. For example, RNA can be isolated,
following standard procedures, from an appropriate cellular
or tissue source. A reverse transcription reaction can be
performed on the RNA using an oligonucleotide primer specific
for the most 5' end of the amplified fragment for the priming
20 of first strand synthesis. The resulting RNA/DNA hybrid can
then be "tailed" with guanines using a standard terminal
transferase reaction, the hybrid can be digested with RNAase
H, and second strand synthesis can then be primed with a
poly-C primer. Thus, cDNA sequences upstream of the
25 amplified fragment can easily be isolated. For a review of
cloning strategies which can be used, see e.g., Sambrook et
al., 1989, Molecular Cloning, A Laboratory Manual, Cold
Springs Harbor Press, N.Y.; and Ausubel et al., 1989, Current
Protocols in Molecular Biology, (Green Publishing Associates
30 and Wiley Interscience, N.Y.).

    As will be appreciated by those skilled in the art, DNA
sequence polymorphisms of a differentially expressed or
pathway gene identified by the methods of the present
invention will exist within a population of individual
35 organisms (e.g., within a human population). Such
polymorphisms may exist, for example, among individuals
within a population due to natural allelic variation. Such

polymorphisms include ones that lead to changes in amino acid
sequence.   An allele is one of a group of genes which occurs
alternatively at a given genetic locus.   Accordingly, as used
herein, an "allelic variant" refers to a nucleotide sequence
5   which occurs at a given locus or to a gene product encoded by
the nucleotide sequence.   Natural allelic variations can
typically result in 1-5% variance in the nucleotide sequence
of a given gene.

Alternative alleles or allelic variants can be
10   identified by sequencing the gene of interest in a number of
different individuals.   This can be readily carried out by
using hybridization probes to identify the same genetic locus
in a variety of individuals.   As used herein, the terms
"gene" and "recombinant gene" refer to nucleic acid molecules
15   comprising an open reading frame encoding a polypeptide of
the invention.   The term can further include nucleic acid
molecules comprising upstream and/or exon/intron sequences
and structure.

With respect to allelic variant of the differentially
20   expressed and pathway genes and gene products of the present
invention, any and all nucleotide variations and/or amino
acid polymorphisms or variations that are the result of
natural allelic variation of the differentially expressed
pathway genes and/or gene products are intended to be within
25   the scope of the present invention.   Such allelic variants
include, but are not limited to, ones that do not alter the
functional activity of a differentially expressed or pathway
gene product of the invention.   Variants also include, but
are not limited to "mutant alleles."   As used herein, a
30   "mutant allele" of a differentially expressed or pathway gene
or gene product of the invention is an allelic variant which
does alter the functional activity of the differentially
expressed or pathway gene product encoded by that gene.

In cases where the differentially expressed or pathway
35   gene identified is the normal, or wild type, gene, this gene
can be used to isolate mutant alleles of the gene.   Such an
isolation is preferable in processes and disorders which are

known or suspected to have a genetic basis. Mutant alleles
can be isolated from individuals either known or suspected to
have a genotype which contributes to TH cell subpopulation-
disorder related symptoms. Mutant alleles and mutant allele
5   products can then be utilized in the therapeutic and
diagnostic assay systems described below.

A cDNA of a mutant gene can be isolated, for example, by
using PCR, a technique which is well known to those of skill
in the art. In this case, the first cDNA strand can be
10 synthesized by hybridizing a oligo-dT oligonucleotide to mRNA
isolated from tissue known to, or suspected of, being
expressed in an individual putatively carrying the mutant
allele, and by extending the new strand with reverse
transcriptase. The second strand of the cDNA is then
15 synthesized using an oligonucleotide that hybridizes
specifically to the 5' end of the normal gene. Using these
two primers, the product is then amplified via PCR, cloned
into a suitable vector, and subjected to DNA sequence
analysis through methods well known to those of skill in the
20 art. By comparing the DNA sequence of the mutant gene to
that of the normal gene, the mutation(s) responsible for the
loss or alteration of function of the mutant gene product can
be ascertained.

Alternatively, a genomic or cDNA library can be
25 constructed and screened using DNA or RNA, respectively, from
a tissue known to or suspected of expressing the gene of
interest in an individual suspected of or known to carry the
mutant allele. The normal gene or any suitable fragment
thereof can then be labeled and used as a probed to identify
30 the corresponding mutant allele in the library. The clone
containing this gene can then be purified through methods
routinely practiced in the art, and subjected to sequence
analysis as described, above, in this Section.

Additionally, an expression library can be constructed
35 utilizing DNA isolated from or cDNA synthesized from a tissue
known to or suspected of expressing the gene of interest in
an individual suspected of or known to carry the mutant

allele. In this manner, gene products made by the putatively
mutant tissue can be expressed and screened using standard
antibody screening techniques in conjunction with antibodies
raised against the normal gene product, as described, below,
5 in Section 5.6. (For screening techniques, see, for example,
Harlow, E. and Lane, eds., 1988, "Antibodies: A Laboratory
Manual", Cold Spring Harbor Press, Cold Spring Harbor.) In
cases where the mutation results in an expressed gene product
with altered function (e.g., as a result of a missense
10 mutation), a polyclonal set of antibodies are likely to
cross-react with the mutant gene product. Library clones
detected via their reaction with such labeled antibodies can
be purified and subjected to sequence analysis as described
in this Section, above.

15   Other allelic variants and/or mutant variants of the
differentially expressed and pathway genes of the invention
include single nucleotide polymorphisms (SNPs), including
biallelic SNPs or biallelic markers which have two alleles,
both of which are present at a fairly high frequency in a
20 population of organisms. Conventional techniques for
detecting SNPs include, e.g., conventional dot blot analysis,
single stranded conformational polymorphism (SSCP) analysis
(see, e.g., Orita et al., 1989, Proc. Natl. Acad. Sci. USA
86:2766-2770), denaturing gradient gel electrophoresis
25 (DGGE), heteroduplex analysis, mismatch cleavage detection,
and other routine techniques well known in the art (see,
e.g., Sheffield et al., 1989, Proc. Natl. Acad. Sci. 86:5855-
5892; Grompe, 1993, Nature Genetics 5:111-117).
Alternative, preferred methods of detecting and mapping SNPs
30 involve microsequencing techniques wherein an SNP site in a
target DNA is detected by a single nucleotide primer
extension reaction (see, e.g., Goelet et al., PCT Publication
No. WO 92/15712; Mundy, U.S. Patent No. 4,656,127; Vary and
Diamond, U.S. Patent No. 4,851,331; Cohen et al., PCT
35 Publication No. WO 91/02087; Chee et al., PCT Publication
No. Wo 95/11995; Landegren et al., 1988, Science 241:1077-
1080; Nicerson et al., 1990, Proc. Natl. Acad. Sci. U.S.A.

87:9823-8927; Pastinen *et al.*, 1997, *Genome Res.* 7:606-614; Pastinen *et al.*, 1996, *Clin. Chem.* 42:1391-1397; Jalanko *et al.*, 1992, *Clin. Chem* 38:39-43; Shumaker *et al.*, 1996, *Hum. Mutation* 7:346-354; Caskey *et al.*, PCT Publication No.
5 95/00669).

5.5. DIFFERENTIALLY EXPRESSED AND PATHWAY GENE PRODUCTS

Differentially expressed and pathway gene products include those proteins encoded by the differentially
10 expressed and pathway genes corresponding to the gene sequences described in Section 5.4, above, as, for example, the peptides listed in FIGS. 9, 17, 22 and 24.

In addition, differentially expressed and pathway gene products can include proteins that represent functionally
15 equivalent gene products. Such gene products include, but are not limited to natural variants of the peptides listed in FIGS. 9, 17, 22 and 24. Such an equivalent differentially expressed or pathway gene product can contain deletions, additions or substitutions of amino acid residues within the
20 amino acid sequence encoded by the differentially expressed or pathway gene sequences described, above, in Section 5.4, but which result in a silent change, thus producing a functionally equivalent differentially expressed or pathway gene product. Amino acid substitutions can be made on the
25 basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and
30 methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.
35 "Functionally equivalent", as utilized herein, refers to a protein capable of exhibiting a substantially similar *in vivo* activity as the endogenous differentially expressed or

pathway gene products encoded by the differentially expressed
or pathway gene sequences described in Section 5.4, above.
Alternatively, when utilized as part of assays such as those
described, below, in Section 5.3, "functionally equivalent"
5 can refer to peptides capable of interacting with other
cellular or extracellular molecules in a manner substantially
similar to the way in which the corresponding portion of the
endogenous differentially expressed or pathway gene product
would.

10   Peptides corresponding to one or more domains of the
differentially expressed or pathway gene products (e.g., TM,
ECD or CD), truncated or deleted differentially expressed or
pathway gene products (e.g., in the case of receptor-type
gene products, proteins in which the full length
15 differentially expressed or pathway gene products, a
differentially expressed or pathway gene peptide or truncated
differentially expressed or pathway gene product is fused to
an unrelated protein are also within the scope of the
invention and can be designed on the basis of the
20 differentially expressd or pathway gene nucleotide and amino
acid sequences disclosed in this Section and in Section 5.4,
above. Such fusion proteins include but are not limited to
IgFC fusions which stabilize the differentially expressed or
pathway gene and prolong half-life in vivo; or fusions to any
25 amino acid sequence that allows the fusion protein to be
anchored to the cell membrane, allowing peptides to be
exhibited on the cell surface; or fusions to an enzyme,
fluorescent protein, or luminescent protein which provide a
marker function.

30   Other mutations to the differentially expressed or
pathway gene product coding sequence can be made to generate
polypeptides that are better suited for expression, scale up,
etc. in the host cells chosen. For example, cysteine
residues can be deleted or substituted with another amino
35 acid in order to eliminate disulfide bridges; in the case of
secreted or transmembrane proteins, N-linked glycosylation
sites can be altered or eliminated to achieve, for example,

expression of a homogeneous product that is more easily
recovered and purified from yeast hosts which are known to
hyperglycosylate N-linked sites. To this end, a variety of
amino acid substitutions at one or both of the first or third
5 amino acid positions of any one or more of the glycosylation
recognition sequences (N-X-S or N-X-T), and/or an amino acid
deletion at the second position of any one or more such
recognition sequences will prevent glycosylation of the
protein at the modified tripeptide sequence. (See, _e.g._,
10 Miyajima et al., 1986, EMBO J. 5(6):1193-1197).

The differentially expressed or pathway gene products of
the invention comprise at least as many continguous amino
acid residues as necessary to represent an epitope fragment
(that is to be recognized by an antibody directed to the
15 differentially expressed or pathway gene product). For
example, such protein fragments or peptides can comprise at
least about 8 contiguous amino acid residues from a full
length differentially expressed or pathway gene product. In
alternative embodiments, the protein fragments and peptides
20 of the invention can comprise about 10, 20, 30, 40, 50, 60,
70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, or more
contiguous amino acid residues of a differentially express or
pathway gene product.

Peptides and/or proteins corresponding to one or more
25 domains of the differentially expressed or pathway gene
products are also within the scope of the present invention.
Further, fusion proteins in which a differentially expressed
or pathway gene product, or a portion thereof such as a
truncated differentially expressed or pathway gene product or
30 a domain of a differentially expressed or pathway gene
product, is fused to an unrelated protein are within the
scope of the present invention. Such proteins and peptides
can be designed on the basis of the differentially expressed
and pathway gene sequences disclosed in Section 5.1 above,
35 and/or on the basis cf the differentially expressed and
pathway gene product sequences disclosed in this section.

Fusion proteins of the invention include, but are not limited to, IgFc fusion proteins which are useful, *e.g.*, to stabilize the differentially expressed or pathway gene product such that the gene product has a prolonged half-life

5 *in vivo*, as well as, *e.g.*, fusions to any amino acid sequence that allows the fusion protein to be anchored to the cell membrane, fusions to an enzyme, fluorescent protein, luminescent protein, or a flag epitope protein or peptide which may be used to provide a marker function.

10   Finally, the differentially expressed and pathway gene products of the present invention also include amino acid sequences encoded by the differentially expressed or pathway genes of the invention wherein domains encoded by one or more exons of the cDNA sequences of those genes, or fragments

15 thereof, have been deleted. The differentially expressed and pathway gene products of the invention can still further comprise post translational modifications, including, but not limited to glycosylations, acetylations, and myrisalations.

The differentially expressed or pathway gene products

20 can be produced by synthetic techniques or via recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the differentially expressed or pathway gene polypeptides and peptides of the invention are described herein. First, the polypeptides and peptides of the

25 invention can be synthesized or prepared by techniques well known in the art. See, for example, Creighton, 1983, "Proteins: Structures and Molecular Principles", W.H. Freeman and Co., N.Y., which is incorporated herein by reference in its entirety. Peptides can, for example, be

30 synthesized on a solid support or in solution.

Alternatively, recombinant DNA methods which are well known to those skilled in the art can be used to construct expression vectors containing differentially expressed or pathway gene protein coding sequences and appropriate

35 transcriptional/translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic

recombination. See, for example, the techniques described in
Sambrook et al., 1989, Molecular Cloning: A Laboratory
Manual, Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
which is incorporated by reference herein in their entirety,

5 and Ausubel, 1989, supra. Alternatively, RNA capable of
encoding differentially expressed or pathway gene protein
sequences can be chemically synthesized using, for example,
synthesizers. See, for example, the techniques described in
"Oligonucleotide Synthesis", 1984, Gait, M.J. ed., IRL Press,

10 Oxford, which is incorporated by reference herein in its
entirety.

    A variety of host-expression vector systems can be
utilized to express the differentially expressed or pathway
gene coding sequences of the invention. Such host-expression

15 systems represent vehicles by which the coding sequences of
interest can be produced and subsequently purified, but also
represent cells which can, when transformed or transfected
with the appropriate nucleotide coding sequences, exhibit the   .
differentially expressed or pathway gene protein of the

20 invention in situ. These include but are not limited to
microorganisms such as bacteria (e.g., E. coli, B. subtilis)
transformed with recombinant bacteriophage DNA, plasmid DNA
or cosmid DNA expression vectors containing differentially
expressed or pathway gene protein coding sequences; yeast

25 (e.g., Saccharomyces, Pichia) transformed with recombinant
yeast expression vectors containing the differentially
expressed or pathway gene protein coding sequences; insect
cell systems infected with recombinant virus expression
vectors (e.g., baculovirus) containing the differentially

30 expressed or pathway gene protein coding sequences; plant
cell systems infected with recombinant virus expression
vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic
virus, TMV) or transformed with recombinant plasmid expres-
sion vectors (e.g., Ti plasmid) containing differentially

35 expressed or pathway gene protein coding sequences; or
mammalian cell systems (e.g. COS, CHO, BHK, 293, 3T3)
harboring recombinant expression constructs containing

promoters derived from the genome of mammalian cells (e.g.,
metallothionein promoter) or from mammalian viruses (e.g.,
the adenovirus late promoter; the vaccinia virus 7.5K
promoter).

5   In bacterial systems, a number of expression vectors can
be advantageously selected depending upon the use intended
for the differentially expressed or pathway gene protein
being expressed. For example, when a large quantity of such
a protein is to be produced, for the generation of antibodies

10 or to screen peptide libraries, for example, vectors which
direct the expression of high levels of fusion protein
products that are readily purified can be desirable. Such
vectors include, but are not limited, to the E. coli
expression vector pUR278 (Ruther et al., 1983, EMBO J.

15 2:1791), in which the differentially expressed or pathway
gene protein coding sequence can be ligated individually into
the vector in frame with the lacZ coding region so that a
fusion protein is produced; pIN vectors (Inouye & Inouye,
1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster,

20 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX
vectors can also be used to express foreign polypeptides as
fusion proteins with glutathione S-transferase (GST). In
general, such fusion proteins are soluble and can easily be
purified from lysed cells by adsorption to glutathione-

25 agarose beads followed by elution in the presence of free
glutathione. The pGEX vectors are designed to include
thrombin or factor Xa protease cleavage sites so that the
cloned target gene protein can be released from the GST
moiety.

30   In an insect system, Autographa californica nuclear
polyhedrosis virus (AcNPV) is used as a vector to express
foreign genes. The virus grows in Spodoptera frugiperda
cells. The differentially expressed or pathway gene coding
sequence can be cloned individually into non-essential

35 regions (for example the polyhedrin gene) of the virus and
placed under control of an AcNPV promoter (for example the
polyhedrin promoter). Successful insertion of differentially

expressed or pathway gene coding sequence will result in
inactivation of the polyhedrin gene and production of non-
occluded recombinant virus (i.e., virus lacking the
proteinaceous coat coded for by the polyhedrin gene). These

5 recombinant viruses are then used to infect Spodoptera
frugiperda cells in which the inserted gene is expressed,
(e.g., see Smith et al., 1983, J. Viol. 46:584; Smith, U.S.
Patent No. 4,215,051).

    In mammalian host cells, a number of viral-based
10 expression systems can be utilized. In cases where an
adenovirus is used as an expression vector, the
differentially expressed or pathway gene coding sequence of
interest can be ligated to an adenovirus
transcription/translation control complex, e.g., the late
15 promoter and tripartite leader sequence. This chimeric gene
can then be inserted in the adenovirus genome by in vitro or
in vivo recombination. Insertion in a non-essential region
of the viral genome (e.g., region E1 or E3) will result in a
recombinant virus that is viable and capable of expressing
20 differentially expressed or pathway gene protein in infected
hosts, (e.g., See Logan & Shenk, 1984, Proc. Natl. Acad. Sci.
USA 81:3655-3659). Specific initiation signals can also be
required for efficient translation of inserted differentially
expressed or pathway gene coding sequences. These signals
25 include the ATG initiation codon and adjacent sequences. In
cases where an entire differentially expressed or pathway
gene, including its own initiation codon and adjacent
sequences, is inserted into the appropriate expression
vector, no additional translational control signals can be
30 needed. However, in cases where only a portion of the
differentially expressed or pathway gene coding sequence is
inserted, exogenous translational control signals, including,
perhaps, the ATG initiation codon, must be provided.
Furthermore, the initiation codon must be in phase with the
35 reading frame of the desired coding sequence to ensure
translation of the entire insert. These exogenous
translational control signals and initiation codons can be of

a variety of origins, both natural and synthetic. The
efficiency of expression can be enhanced by the inclusion of
appropriate transcription enhancer elements, transcription
terminators, etc. (see Bittner et al., 1987, Methods in
5 Enzymol. 153:516-544).

In addition, a host cell strain can be chosen which
modulates the expression of the inserted sequences, or
modifies and processes the gene product in the specific
fashion desired. Such modifications (e.g., glycosylation)
10 and processing (e.g., cleavage) of protein products can be
important for the function of the protein. Different host
cells have characteristic and specific mechanisms for the
post-translational processing and modification of proteins.
Appropriate cell lines or host systems can be chosen to
15 ensure the correct modification and processing of the foreign
protein expressed. To this end, eukaryotic host cells which
possess the cellular machinery for proper processing of the
primary transcript, glycosylation, and phosphorylation of the
gene product can be used. Such mammalian host cells include
20 but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293,
3T3, WI38, etc.

For long-term, high-yield production of recombinant
proteins, stable expression is preferred. For example, cell
lines which stably express the differentially expressed or
25 pathway gene protein can be engineered. Rather than using
expression vectors which contain viral origins of
replication, host cells can be transformed with DNA
controlled by appropriate expression control elements (e.g.,
promoter, enhancer, sequences, transcription terminators,
30 polyadenylation sites, etc.), and a selectable marker.
Following the introduction of the foreign DNA, engineered
cells can be allowed to grow for 1-2 days in an enriched
media, and then are switched to a selective media. The
selectable marker in the recombinant plasmid confers
35 resistance to the selection and allows cells to stably
integrate the plasmid into their chromosomes and grow to form
foci which in turn can be cloned and expanded into cell

lines. This method can advantageously be used to engineer
cell lines which express the differentially expressed or
pathway gene protein. Such engineered cell lines can be
particularly useful in screening and evaluation of compounds
5 that affect the endogenous activity of the differentially
expressed or pathway gene protein.

A number of selection systems can be used, including but
not limited to the herpes simplex virus thymidine kinase
(Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine
10 phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc.
Natl. Acad. Sci. USA 48:2026), and adenine
phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817)
genes can be employed in tk⁻, hgprt⁻ or aprt⁻ cells,
respectively. Also, antimetabolite resistance can be used as
15 the basis of selection for dhfr, which confers resistance to
methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA
77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA
78:1527); gpt, which confers resistance to mycophenolic acid
(Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072);
20 neo, which confers resistance to the aminoglycoside G-418
(Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and
hygro, which confers resistance to hygromycin (Santerre, et
al., 1984, Gene 30:147) genes.

Alternatively, any fusion protein may be readily
25 purified by utilizing an antibody specific for the fusion
protein being expressed. For example, a system described by
Janknecht et al. allows for the ready purification of non-
denatured fusion proteins expressed in human cells lines
(Janknecht, et al., 1991, Proc. Natl. Acad. Sci. USA 88:
30 8972-8976). In this system, the gene of interest is
subcloned into a vaccinia recombination plasmid such that the
gene's open reading frame is translationally fused to an
amino-terminal tag consisting of six histidine residues.
Extracts from cells infected with recombinant vaccinia virus
35 are loaded onto Ni^{2+}-nitriloacetic acid-agarose columns and
histidine-tagged proteins are selectively eluted with
imidazole-containing buffers.

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When used as a component in assay systems such as those
described herein, the differentially expressed or pathway
gene protein can be labeled, either directly or indirectly,
to facilitate detection of a complex formed between the
5 differentially expressed or pathway gene protein and a test
substance. Any of a variety of suitable labeling systems can
be used including but not limited to radioisotopes such as
^{125}I; enzyme labelling systems that generate a detectable
colorimetric signal or light when exposed to substrate; and
10 fluorescent labels.

     Indirect labeling involves the use of a protein, such as
a labeled antibody, which specifically binds to either a
differentially expressed or pathway gene product. Such
antibodies include but are not limited to polyclonal,
15 monoclonal, chimeric, single chain, Fab fragments and
fragments produced by an Fab expression library.

     Where recombinant DNA technology is used to produce the
differentially expressed or pathway gene protein for such
assay systems, it can be advantageous to engineer fusion
20 proteins that can facilitate labeling (either direct or
indirect), immobilization, solubility and/or detection.

     Fusion proteins, which can facilitate solubility and/or
expression, and can increase the blood half-life of the
protein, can include, but are not limited to soluble Ig-
25 tailed fusion proteins. Methods for engineering such soluble
Ig-tailed fusion proteins are well known to those of skill in
the art. See, for example U.S. Patent No. 5,116,964, which
is incorporated herein by reference in its entirety.
Further, in addition to the Ig-region encoded by the IgG1
30 vector, the Fc portion of the Ig region utilized can be
modified, by amino acid substitutions, to reduce complement
activation and Fc binding. (See, e.g., European Patent No.
239400 B1, August 3, 1994).

     Among the soluble Ig-tailed fusion proteins which can be
35 produced are soluble Ig-tailed fusion proteins containing 103
gene products, 200 gene products or 10 gene products. The
103 gene product or 200 gene contained within such fusion

proteins can comprise, respectively, for example, the 103
gene extracellular or secreted domain or portions, preferably
ligand-binding portions, thereof, or the 200 gene
extracellular domain or portions, preferably ligand-binding
5 portions, thereof. The 10 gene product contained within such
fusion proteins can comprise, for example, one or more of the
extracellular domains or portions, preferably ligand-binding
portions, of the seven transmembrane domain sequence motif.

The amino acid sequences of the secreted and membrane
10 bound forms of the murine 103 gene products are known.
Further, the amino acid sequence of a soluble human 103 gene
product is known. (See, for example, Klemenz, R. et al.,
1989, Proc. Natl. Acad. Sci. USA 86:5708-5712; Tominaga, S.,
1989, FEBS Lett. 258:301-301; Werenskiold, A.K. et al., 1989,
15 Mol. Cell. Biol.9:5207-5214; Tominaga, S. et al., 1992,
Biochem. Biophys. Acta. 1171:215-218; Werenskiold, A.K.,
1992, Eur. J. Biochem. 204:1041-1047; Yanagisawa, K. et al.,
1993, FEBS Lett. 318:83-87; Bergers, G. et al., 1994, EMBO J.
13:1176-1188; and Kumar, S. 1997, Biochem Biophys Res Commun
20 235:474-478.)

Further, as indicated in FIG. 4B, the amino acid
residues which delineate the extracellular, transmembrane and
cytoplasmic domains of the murine 103 gene products are also
known. Still further, the amino acid sequences of murine and
25 human 103 gene products are listed in SEQ ID NOS: 39 (murine
full length, transmembrane 103 gene product), 41 (murine
extracellular domain of the full length, transmembrane
product, plus amino terminal signal peptide), 43 (murine
intracellular domain of the full length, transmembrane
30 product), 48 (murine transmembrane domain of the full
length, transmembrane product), 47 (murine secreted 103 gene
product), and 45 (human secreted/extracellular 103 gene
product domain, plus amino terminal signal peptide).

The murine 103 gene encodes two mRNA products, a 2.5 Kb
35 transcript and a 4.5 Kb transcript, which correspond to the
soluble and membrane forms of the 103 protein (Kumar, S.
1997, Biochem Biophys Res Commun 235:474-478). In contrast,

the human 103 gene encodes three mRNA products, an
approximately 4.2 Kb transcript, an approximately 2.5 Kb
transcript, and an approximately 1.4 Kb transcript (Kumar, S.
1997, Biochem Biophys Res Commun 235:474-478). Nucleotide
5 sequences encoding 103 gene products are listed herein at SEQ
ID NOS: 38 (nucleotide sequence encoding murine full length,
transmembrane 103 gene product), 40 (nucleotide sequence
encoding extracellular domain of the murine 103 transmembrane
gene product, plus amino terminal signal peptide), 42
10 (nucleotide sequence encoding the intracellular domain of the
murine 103 transmembrane gene product), 46 (nucleotide
sequence encoding the transmembrane domain of the murine 103
transmembrane gene product), 49 (nucleotide sequence encoding
the secreted murine 103 gene product), and 44 (nucleotide
15 sequence encoding human secreted/extracellular 103 gene
product domain, plus amino terminal signal peptide). Murine
and human 103 amino acid and nucleotide sequences are also
depicted in FIG. 4C, FIG. 4D, FIG. 4E, FIG. 4F, FIG. 4G, and
FIG. 4H. The nucleotide sequences of the 4.2 Kb and 2.5 Kb
20 human 103 gene transcripts, as well as the amino acid
sequences encoded by these transcripts, can be obtained
utilizing standard techniques well known to those of skill in
the art, including, _e.g._, techniques such as those discussed
herein.
25   Therefore, by utilizing well known techniques, one of
skill in the art would readily be capable of producing such
soluble Ig-tailed 103 gene product fusion proteins. The
Example presented below, in Section 10, below, describes the
construction of a 103 gene product-Ig fusion protein.
30   The signal sequence, extracellular, transmembrane and
cytoplasmic domains of both the murine and human 200 gene
products have been elucidated and can be utilized in, for
example, the construction of 200 gene product-Ig fusion
proteins. Specifically, the 280 amino acid murine 200 gene
35 product (FIG. 17; SEQ ID NO:10) contains a signal sequence
from approximately amino acid residue 1 to approximately
amino acid residue 20, an extracellular domain from

approximately amino acid residue 21 to approximately amino
acid residue 192, a transmembrane domain from approximately
amino acid residue 193 to amino acid residue 214, and a
cytoplasmic domain from approximately amino acid residue 215

5 to amino acid residue 280. Further, the 301 amino acid human
200 gene product (FIG. 24; SEQ. ID. NO: 24) contains a signal
sequence from amino acid residue 1 to approximately 20, a
mature extracellular domain from approximately amino acid
residue 21 to 200, a transmembrane domain from approximately

10 amino acid residue 201-224 and a cytoplasmic domain from
approximately amino acid residue 225 to 301. Given the
elucidation of these domains, one of skill in the art would
readily be capable of producing soluble Ig-tailed 200 gene
product fusion proteins. The Example presented, below, in

15 Section 10 describes the construction of murine and human 200
gene product-Ig fusion proteins.

The 338 amino acid residue 10 gene product (FIG.9, SEQ
ID NO:9) extracellular domains include 10 gene product amino
acid residues from approximately amino acid residue 1 to 19,

20 approximately amino acid residue 74 to 87, approximately
amino acid residue 153 to 187 and approximately amino acid
residue 254 to 272. Thus, such 10 gene product domain
information can be used, in conjunction with well-known
techniques, such that one of skill in the art can readily be

25 capable of producing soluble Ig-tailed 10 gene fusion
proteins comprising one or more 10 gene product extra-
cellular domain regions and an Ig tail.

5.6. ANTIBODIES SPECIFIC FOR DIFFERENTIALLY EXPRESSED OR PATHWAY GENE PRODUCTS

30
Described herein are methods for the production of
antibodies capable of specifically recognizing one or more
differentially expressed or pathway gene product epitopes.
Such antibodies can include, but are not limited to,

35 polyclonal antibodies, monoclonal antibodies (mAbs),
humanized or chimeric antibodies, single chain antibodies,
Fab fragments, F(ab')$_2$ fragments, fragments produced by a Fab

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expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The Ig tails of such antibodies can be modified to reduce complement activation and Fc binding. (See, for example, European
5 Patent No. 239400 B1, August 3, 1994).

Such antibodies can be used, for example, in the detection of a fingerprint, target, or pathway gene product in a biological sample, and can be used as part of diagnostic techniques. Alternatively, such antibodies can be utilized
10 as part of an immune disorder treatment method, as described, below, in Section 5.9. For example, the antibodies can be used to modulate target gene activity, can be used to modulate TH cell subpopulation differentiation, maintenance and/or effector function, or, in the case of antibodies
15 directed to cell surface epitopes, can be used to isolate a TH cell subpopulation of interest, for either depletion or augmentation purposes.

Such antibodies can also be utilized as part of a method for treatment of an ischemic disorder or injury, as described
20 in Section 5.10.3.1, below. For example, the antibodies can be used to block or inhibit activity of one or more of the gene products of the invention, thereby reducing or inhibiting repair of certain ischemic tissues, for example carcinogenic tumors.

25   For the production of antibodies to a differentially expressed or pathway gene, various host animals can be immunized by injection with a differentially expressed or pathway gene protein, or a portion thereof. Such host animals can include but are not limited to rabbits, mice, and
30 rats, to name but a few. Various adjuvants can be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols,
35 polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human

adjuvants such as BCG (bacille Calmette-Guerin) and
Corynebacterium parvum.

    Polyclonal antibodies are heterogeneous populations of
antibody molecules derived from the sera of animals immunized
5 with an antigen, such as target gene product, or an
antigenic functional derivative thereof. For the production
of polyclonal antibodies, host animals such as those
described above, can be immunized by injection with
differentially expressed or pathway gene product supplemented
10 with adjuvants as also described above.

    Monoclonal antibodies, which are homogeneous populations
of antibodies to a particular antigen, can be obtained by any
technique which provides for the production of antibody
molecules by continuous cell lines in culture. These
15 include, but are not limited to the hybridoma technique of
Kohler and Milstein, (1975, Nature 256:495-497; and U.S.
Patent No. 4,376,110), the human B-cell hybridoma technique
(Kosbor et al., 1983, Immunology Today 4:72; Cole et al.,
1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-
20 hybridoma technique (Cole et al., 1985, Monoclonal Antibodies
And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such
antibodies can be of any immunoglobulin class including IgG,
IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma
producing the mAb of this invention can be cultivated in
25 vitro or in vivo. Production of high titers of mAbs in vivo
makes this the presently preferred method of production.

    In addition, techniques developed for the production of
"chimeric antibodies" (Morrison et al., 1984, Proc. Natl.
Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature,
30 312:604-608; Takeda et al., 1985, Nature, 314:452-454; U.S.
Patent No. 4,816,567) by splicing the genes from a mouse
antibody molecule of appropriate antigen specificity together
with genes from a human antibody molecule of appropriate
biological activity can be used. A chimeric antibody is a
35 molecule in which different portions are derived from
different animal species, such as those having a variable

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region derived from a murine mAb and a human immunoglobulin constant region.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird,
5 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) and for making humanized monoclonal antibodies (U.S. Patent No. 5,225,539, which is incorporated herein by reference in its entirety) can be utilized to produce anti-
10 differentially expressed or anti-pathway gene product antibodies.

Antibody fragments which recognize specific epitopes can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')$_2$
15 fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')$_2$ fragments. Alternatively, Fab expression libraries can be constructed (Huse et al., 1989, Science, 246:1275-1281) to
20 allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibodies to the differentially expressed or pathway gene products can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" such gene products, using
25 techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, 1993, FASEB J 7(5):437-444; and Nissinoff, 1991, J. Immunol. 147(8):2429-2438). For example, in the case of receptor-type molecules (e.g., 10, 103 and 200 gene products) antibodies which bind to the ECD and
30 competitively inhibit the binding of ligand to the receptor can be used to generate anti-idiotypes that "mimic" the ECD and, therefore, bind and neutralize the ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens of TH cell
35 subpopulation-related disorders.

Production of antibodies directed against the extracellular domain of the 103 gene product are described in

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Section 12, below. Also, production of antibodies directed against the extracellular domain of the 200 gene product are described in Section 13, below.

5   5.7.   CELL-AND ANIMAL-BASED MODEL SYSTEMS
           Described herein are cell- and animal-based systems which act as models for immune disorders and for models of TH cell subpopulation differentiation, maintenance, and/or effector function. These systems can be used in a variety of
10 applications. For example, the animal-based model systems can be utilized to identify differentially expressed genes via the in vivo paradigm described, above, in Section 5.1.1.1. Cell- and animal-based model systems can also be used to further characterize differentially expressed and
15 pathway genes, as described, above, in Section 5.3. Such further characterization can, for example, indicate that a differentially expressed gene is a target gene. Second, such assays can be utilized as part of screening strategies designed to identify compounds which are capable of
20 ameliorating TH cell subpopulation-related disorder symptoms, as described, below. Thus, the animal- and cell-based models can be used to identify drugs, pharmaceuticals, therapies and interventions which can be effective in treating immune disorders such as TH cell subpopulation-related disorders.
25 In addition, as described in detail, below, in Section 5.11.1, such animal models can be used to determine the LD_{50} and the ED_{50} in animal subjects, and such data can be used to determine the in vivo efficacy of potential immune disorder treatments.
30

           5.7.1 ANIMAL-BASED SYSTEMS
           Animal-based model systems of TH cell subpopulation-related disorders can include both non-recombinant animals as well as recombinantly engineered transgenic animals.
35   Animal models for TH cell subpopulation-related disorders can include, for example, genetic models. For example, such animal models can include Leishmania resistance

models, experimental allergic encephalomyelitis models and
(BALB/c Cr x DBA/2Cr) F1 mice. These latter mice develop a
fatal disseminated disease by systemic infection with
virulent <u>Candida albicans</u> associated with strong TH2-like
5 responses. Additionally, well known mouse models for asthma
can be utilized to study the amelioration of symptoms caused
by a TH2-like response. (See, for example, Lukacs, N.W. et
al., 1994, Am. J. Resp. Cell Mol. Biol. <u>10</u>:526-532; Gavett,
S.H. et al., 1994, Am. J. Resp. Cell Mol. Biol. <u>10</u>:587-593.)
10 Further, the animal model, murine acquired immunodeficiency
syndrome (MAIDS; Kanagawa, B. et al., 1993, Science <u>262</u>:240;
Makino, M. et al., 1990, J. Imm. <u>144</u>:4347) can be used for
such studies.

Alternatively, such well known animal models as SCIDhu
15 mice (see for example, Kaneshima, H. et al., 1994, Curr.
Opin. Imm. <u>6</u>:327-333) which represents an in vivo model of
the human hematolymphoid system, can be utilized. Further,
the RAG-2-deficient blastocyst complementation technique
(Chen, J. et al., 1993, Proc. Natl. Acad. Sci. USA <u>90</u>:4528-
20 4532; Shinkai, Y. et al., 1992, Cell <u>68</u>:855-867) can be
utilized to produce mice containing, for example, humanized
lymphocytes and/or which express target gene sequences.
Still further, targeting techniques directed specifically to
T cells, for example, the technique of Gu et al. (Gu, H. et
25 al., 1994, Science <u>265</u>:103-106) can be utilized to produce
animals containing transgenes in only T cell populations.

Further, animal models such as the adoptive transfer
model described, <u>e.g.</u>, in Cohn, L. et al., 1997, J. Exp. Med.
<u>186</u>:1737-1747) and described and utilized in Section 12,
30 below, can be used. In such an animal system, aeroallergen
provocation of TH1 or TH2 recipient mice results in TH
effector cell migration to the airways and is associated with
an intense neutrophilic (TH1) and eosinophilic (TH2) lung
mucosal inflammatory response. The animal model represents
35 an accepted model for the TH2-like disorder asthma.

Animal models exhibiting TH cell subpopulation-related
disorder-like symptoms can be engineered by utilizing, for

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example, target gene sequences such as those described,
above, in Section 5.4, in conjunction with techniques for
producing transgenic animals that are well known to those of
skill in the art. For example, target gene sequences can be
5 introduced into, and overexpressed and/or misexpressed in,
the genome of the animal of interest, or, if endogenous
target gene sequences are present, they can either be
overexpressed, misexpressed, or, alternatively, can be
disrupted in order to underexpress or inactivate target gene
10 expression. The construction and characterization of 200
gene and 103 gene transgenic animals is described in Section
11, below.

     In order to overexpress or misexpress a target gene
sequence, the coding portion of the target gene sequence can
15 be ligated to a regulatory sequence which is capable of
driving high level gene expression or expression in a cell
type in which the gene is not normally expressed in the
animal and/or cell type of interest. Such regulatory regions
will be well known to those of skill in the art, and can be
20 utilized in the absence of undue experimentation.

     For underexpression of an endogenous target gene
sequence, such a sequence can be isolated and engineered such
that when reintroduced into the genome of the animal of
interest, the endogenous target gene alleles will be
25 inactivated. Preferably, the engineered target gene sequence
is introduced via gene targeting such that the endogenous
target sequence is disrupted upon integration of the
engineered target gene sequence into the animal's genome.
Gene targeting is discussed, below, in this Section.

30   Animals of any species, including. but not limited to,
mice, rats, rabbits, guinea pigs, pigs, micro-pigs, goats,
and non-human primates, e.g., baboons, squirrels, monkeys,
and chimpanzees can be used to generate animal models of TH
cell subpopulation-related disorders.

35   Any technique known in the art can be used to introduce
a target gene transgene into animals to produce the founder
lines of transgenic animals. Such techniques include, but

are not limited to pronuclear microinjection (Hoppe, P.C. and
Wagner, T.E., 1989, U.S. Pat. No. 4,873,191); retrovirus
mediated gene transfer into germ lines (Van der Putten et
al., 1985, Proc. Natl. Acad. Sci., USA 82:6148-6152); gene
5   targeting in embryonic stem cells (Thompson et al., 1989,
Cell 56:313-321); electroporation of embryos (Lo, 1983, Mol
Cell. Biol. 3:1803-1814); and sperm-mediated gene transfer
(Lavitrano et al., 1989, Cell 57:717-723); etc. For a review
of such techniques, see Gordon, 1989, Transgenic Animals,
10 Intl. Rev. Cytol. 115:171-229, which is incorporated by
reference herein in its entirety.

    The present invention provides for transgenic animals
that carry the transgene in all their cells, as well as
animals which carry the transgene in some, but not all their
15 cells, i.e., mosaic animals. (See, for example, techniques
described by Jakobovits, 1994, Curr. Biol. 4:761-763.) The
transgene can be integrated as a single transgene or in
concatamers, e.g., head-to-head tandems or head-to-tail
tandems. The transgene can also be selectively introduced
20 into and activated in a particular cell type by following,
for example, the teaching of Lasko et al. (Lasko, M. et al.,
1992, Proc. Natl. Acad. Sci. USA 89:6232-6236). The
regulatory sequences required for such a cell-type specific
activation will depend upon the particular cell type of
25 interest, and will be apparent to those of skill in the art.
    When it is desired that the target gene transgene be
integrated into the chromosomal site of the endogenous target
gene, gene targeting is preferred. Briefly, when such a
technique is to be utilized, vectors containing some
30 nucleotide sequences homologous to the endogenous target gene
of interest are designed for the purpose of integrating, via
homologous recombination with chromosomal sequences, into and
disrupting the function of, the nucleotide sequence of the
endogenous target gene. The transgene can also be
35 selectively introduced into a particular cell type, thus
inactivating the endogenous gene of interest in only that
cell type, by following, for example, the teaching of Gu et

al. (Gu, H. et al., 1994, Science 265:103-106). The
regulatory sequences required for such a cell-type specific
inactivation will depend upon the particular cell type of
interest, and will be apparent to those of skill in the art.

5   Once transgenic animals have been generated, the
expression of the recombinant target gene and protein can be
assayed utilizing standard techniques. Initial screening can
be accomplished by Southern blot analysis or PCR techniques
to analyze animal tissues to assay whether integration of the

10 transgene has taken place. The level of mRNA expression of
the transgene in the tissues of the transgenic animals can
also be assessed using techniques which include but are not
limited to Northern blot analysis of tissue samples obtained
from the animal, in situ hybridization analysis, and RT-PCR.

15 Samples of target gene-expressing tissue, can also be
evaluated immunocytochemically using antibodies specific for
the target gene transgene gene product of interest.

       The target gene transgenic animals that express target
gene mRNA or target gene transgene peptide (detected

20 immunocytochemically, using antibodies directed against
target gene product epitopes) at easily detectable levels can
then be further evaluated to identify those animals which
display characteristic TH cell subpopulation-related
disorder-like symptoms, or exhibit characteristic TH cell

25 subpopulation differentiation phenotypes. TH1-like-related
disorder symptoms can include, for example, those associated
with chronic inflammatory diseases and disorders, such as
Crohn's disease, reactive arthritis, including Lyme disease,
insulin-dependent diabetes, organ-specific autoimmunity,

30 including multiple sclerosis, Hashimoto's thyroiditis and
Grave's disease, contact dermatitis, psoriasis, graft
rejection, graft versus host disease and sarcoidosis. TH2-
like-related disorder symptoms can include, those associated
with atopic conditions, such as asthma and allergy, including

35 allergic rhinitis, gastrointestinal allergies, including food
allergies, eosinophilia, conjunctivitis, glomerular
nephritis, certain pathogen susceptibilities such as

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helminthic (e.g., leishmaniasis) and certain viral
infections, including HIV, and bacterial infections,
including tuberculosis and lepromatous leprosy.

　　　　Additionally, specific cell types within the transgenic
5　animals can be analyzed and assayed for cellular phenotypes
characteristic of TH cell subpopulation-related disorders.
Such cellular phenotypes can include, for example,
differential cytokine expression characteristic of the TH
cell subpopulation of interest. Further, such cellular
10　phenotypes can include an assessment of a particular cell
type's fingerprint pattern of expression and its comparison
to known fingerprint expression profiles of the particular
cell type in animals exhibiting specific TH cell
subpopulation-related disorders. Such transgenic animals
15　serve as suitable model systems for TH cell-related
disorders.

　　　　Once target gene transgenic founder animals are produced
(i.e., those animals which express target gene proteins in
cells or tissues of interest, and which, preferably, exhibit
20　symptoms of TH cell subpopulation-related disorders), they
can be bred, inbred, outbred, or crossbred to produce
colonies of the particular animal. Examples of such breeding
strategies include but are not limited to: outbreeding of
founder animals with more than one integration site in order
25　to establish separate lines; inbreeding of separate lines in
order to produce compound target gene transgenics that
express the target gene transgene of interest at higher
levels because of the effects of additive expression of each
target gene transgene; crossing of heterozygous transgenic
30　animals to produce animals homozygous for a given integration
site in order to both augment expression and eliminate the
possible need for screening of animals by DNA analysis;
crossing of separate homozygous lines to produce compound
heterozygous or homozygous lines; breeding animals to
35　different inbred genetic backgrounds so as to examine effects
of modifying alleles on expression of the target gene
transgene and the development of TH cell subpopulation-

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related disorder-like symptoms. One such approach is to
cross the target gene transgenic founder animals with a wild
type strain to produce an F1 generation that exhibits TH cell
subpopulation-related disorder-like symptoms, such as those
5 described above. The F1 generation can then be inbred in
order to develop a homozygous line, if it is found that
homozygous target gene transgenic animals are viable.

5.7.2. CELL-BASED ASSAYS

10   Cells that contain and express target gene sequences
which encode target gene protein, and, further, exhibit
cellular phenotypes associated with a TH cell subpopulation-
related disorder of interest, can be utilized to identify
compounds that exhibit an ability to ameliorate TH cell
15 subpopulation-related disorder symptoms. Cellular phenotypes
which can indicate an ability to ameliorate TH cell
subpopulation-related disorder symptoms can include, for
example, an inhibition or potentiation of cytokine or cell
surface marker expression associated with the TH cell
20 subpopulation of interest, or, alternatively, an inhibition
or potentiation of specific TH cell subpopulations.
        Further, the fingerprint pattern of gene expression of
cells of interest can be analyzed and compared to the normal,
non-TH cell subpopulation-related disorder fingerprint
25 pattern. Those compounds which cause cells exhibiting TH
cell subpopulation-related disorder-like cellular phenotypes
to produce a fingerprint pattern more closely resembling a
normal fingerprint pattern for the cell of interest can be
considered candidates for further testing regarding an
30 ability to ameliorate TH cell subpopulation-related disorder
symptoms.
        Cells which can be utilized for such assays can, for
example, include non-recombinant cell lines, such as Dorris,
AE7, D10.G4, DAX, D1.1 and CDC25 cell lines. In addition,
35 purified primary naive T cells derived from either transgenic
or non-transgenic strains can also be used.

Further, cells which can be used for such assays can also include recombinant, transgenic cell lines. For example, the TH cell subpopulation-related disorder animal models of the invention, discussed, above, in Section 5.7.1,

5 can be used to generate, for example, TH1-like and/or TH2-like cell lines that can be used as cell culture models for the disorder of interest. While primary cultures derived from TH cell subpopulation-related disorder transgenic animals can be utilized, the generation of continuous cell

10 lines is preferred. For examples of techniques which can be used to derive a continuous cell line from the transgenic animals, see Small et al., 1985, Mol. Cell Biol. 5:642-648.

Alternatively, cells of a cell type known to be involved in TH cell subpopulation-related disorders can be transfected

15 with sequences capable of increasing or decreasing the amount of target gene expression within the cell. For example, target gene sequences can be introduced into, and overexpressed in, the genome of the cell of interest, or, if endogenous target gene sequences are present, they can either

20 be overexpressed or, alternatively, can be disrupted in order to underexpress or inactivate target gene expression.

In order to overexpress a target gene sequence, the coding portion of the target gene sequence can be ligated to a regulatory sequence which is capable of driving gene

25 expression in the cell type of interest. Such regulatory regions will be well known to those of skill in the art, and can be utilized in the absence of undue experimentation.

For underexpression of an endogenous target gene sequence, such a sequence can be isolated and engineered such

30 that when reintroduced into the genome of the cell type of interest, the endogenous target gene alleles will be inactivated. Preferably, the engineered target gene sequence is introduced via gene targeting such that the endogenous target sequence is disrupted upon integration of the

35 engineered target gene sequence into the cell's genome. Gene targeting is discussed, above, in Section 5.7.1.

Transfection of target gene sequence nucleic acid can be accomplished by utilizing standard techniques. See, for example, Ausubel, 1989, supra. Transfected cells should be evaluated for the presence of the recombinant target gene

5 sequences, for expression and accumulation of target gene mRNA, and for the presence of recombinant target gene protein production. In instances wherein a decrease in target gene expression is desired, standard techniques can be used to demonstrate whether a decrease in endogenous target gene

10 expression and/or in target gene product production is achieved.

Cells to be utilized can, for example, be stimulated or activated as, described e.g., in the Examples presented below.

15

5.8. SCREENING ASSAYS FOR COMPOUNDS THAT INTERACT WITH THE TARGET GENE PRODUCT

The following assays are designed to identify compounds that bind to target gene products, bind to other cellular

20 proteins that interact with a target gene product, and to compounds that interfere with the interaction of the target gene product with other cellular proteins. For example, in the cases of 10, 103 and 200 gene products, which are or are predicted to be transmembrane receptor-type proteins, such

25 techniques can identify ligands for such receptors. A compound which binds a 103 gene product (a 103 gene product ligand, for example) can act as the basis for amelioration of such TH2-like-specific disorders as asthma or allergy, given that gene 103 expression is TH2-specific. A 200 gene product

30 ligand can, for example, act as the basis for amelioration of TH1-like-specific disorders. A 10 gene product ligand can, for example, act as the basis for amelioratoin of a wide range of T cell disorders, given the TH inducible nature of it gene expression pattern. Any such binding compound can

35 act as a marker for the presence of TH cell subpopulations. For example, a compound which binds the 103 gene product can

act as a marker, for example, a diagnostic marker, for TH2 cells, e.g., for TH2 cell differentiation.

Compounds can include, but are not limited to, other cellular proteins. Further, such compounds can include, but
5 are not limited to, peptides such as, for example, soluble peptides, including, but not limited to, Ig-tailed fusion peptides, comprising extracellular portions of target gene product transmembrane receptors, and members of random peptide libraries (see, e.g., Lam, K.S. et al., 1991, Nature
10 354:82-84; Houghten, R. et al., 1991, Nature 354:84-86) made of D-and/or L-configuration amino acids, phosphopeptides (including but not limited to members of random or partially degenerate, directed phosphopeptide libraries; see, e.g., Songyang, Z. et al., 1993, Cell 72:767-778), antibodies
15 (including, but not limited to polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, F(ab')$_2$ and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules. In the case of receptor-type
20 target molecules, such compounds can include organic molecules (e.g., peptidomimetics) that bind to the ECD and either mimic the activity triggered by the natural ligand (i.e., agonists); as well as peptides, antibodies or fragments thereof, and other organic compounds that mimic the
25 ECD (or a portion thereof) and bind to a "neutralize" natural ligand.

Computer modelling and searching technologies permit identification of compounds, or the improvement of already identified compounds, that can modulate target or pathway
30 gene expression or activity. Having identified such a compound or composition, the active sites or regions are identified.

In the case of compounds affecting receptor molecules, such active sites might typically be ligand binding sites,
35 such as the interaction domains of ligand with receptor itself. The active site can be identified using methods known in the art including, for example, from the amino acid

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sequences of peptides, from the nucleotide sequences of
nucleic acids, or from study of complexes of the relevant
compound or composition with its natural ligand. In the
latter case, chemical or X-ray crystallographic methods can
5 be used to find the active site by finding where on the
factor the complexed ligand is found.

Next, the three dimensional geometric structure of the
active site is determined. This can be done by known
methods, including X-ray crystallography, which can determine
10 a complete molecular structure. On the other hand, solid or
liquid phase NMR can be used to determine certain intra-
molecular distances. Any other experimental method of
structure determination can be used to obtain partial or
complete geometric structures. The geometric structures may
15 be measured with a complexed ligand, natural or artificial,
which may increase the accuracy of the active site structure
determined.

If an incomplete or insufficiently accurate structure is
determined, the methods of computer based numerical modelling
20 can be used to complete the structure or improve its
accuracy. Any recognized modelling method may be used,
including parameterized models specific to particular
biopolymers such as proteins or nucleic acids, molecular
dynamics models based on computing molecular motions,
25 statistical mechanics models based on thermal ensembles, or
combined models. For most types of models, standard
molecular force fields, representing the forces between
constituent atoms and groups, are necessary, and can be
selected from force fields known in physical chemistry. The
30 incomplete or less accurate experimental structures can serve
as constraints on the complete and more accurate structures
computed by these modeling methods.

Finally, having determined the structure of the active
site, either experimentally, by modeling, or by a
35 combination, candidate modulating compounds can be identified
by searching databases containing compounds along with
information on their molecular structure. Such a search seeks

compounds having structures that match the determined active
site structure and that interact with the groups defining the
active site. Such a seach can be manual, but is preferably
computer assisted. These compounds found from this search
5 are potential target or pathway gene product modulating
compounds.

Alternatively, these methods can be used to identify
improved modulating compounds from an already known
modulating compound or ligand. The composition of the known
10 compound can be modified and the structural effects of
modification can be determined using the experimental and
computer modelling methods described above applied to the new
composition. The altered structure is then compared to the
active site structure of the compound to determine if an
15 improved fit or interaction results. In this manner
systematic variations in composition, such as by varying side
groups, can be quickly evaluated to obtain modified
modulating compounds or ligands of improved specificity or
activity.

20   Further experimental and computer modeling methods
useful to identify modulating compounds based upon
identification of the active sites of target or pathway gene
or gene products and related transduction and transcription
factors will be apparent to those of skill in the art.

25   Examples of molecular modelling systems are the CHARMm
and QUANTA programs (Polygen Corporation, Waltham, MA).
CHARMm performs the energy minimization and molecular
dynamics functions. QUANTA performs the construction,
graphic modelling and analysis of molecular structure.
30 QUANTA allows interactive construction, modification,
visualization, and analysis of the behavior of molecules with
each other.

A number of articles review computer modelling of drugs
interactive with specific proteins, such as Rotivinen, et
35 al., 1988, Acta Pharmaceutical Fennica 97:159-166; Ripka, New
Scientist 54-57 (June 16, 1988); McKinaly and Rossmann, 1989,
Annu. Rev. Pharmacol. Toxiciol. 29:111-122; Perry and Davies,

QSAR: Quantitative Structure-Activity Relationships in Drug
Design pp. 189-193 (Alan R. Liss, Inc. 1989); Lewis and
Dean, 1989 Proc. R. Soc. Lond. 236:125-140 and 1-162; and,
with respect to a model receptor for nucleic acid components,
5 Askew, et al., 1989, J. Am. Chem. Soc. 111:1082-1090. Other
computer programs that screen and graphically depict
chemicals are available from companies such as BioDesign,
Inc. (Pasadena, CA.), Allelix, Inc. (Mississauga, Ontario,
Canada), and Hypercube, Inc. (Cambridge, Ontario). Although
10 these are primarily designed for application to drugs
specific to particular proteins, they can be adapted to
design of drugs specific to regions of DNA or RNA, once that
region is identified.

     Although generally described above with reference to
15 design and generation of compounds which could alter binding,
one could also screen libraries of known compounds, including
natural products or synthetic chemicals, and biologically
active materials, including proteins, for compounds which are
inhibitors or activators.
20   Compounds identified via assays such as those described
herein can be useful, for example, in elaborating the
biological function of the target gene product, and for
ameliorating the symptoms of immune disorders. In instances,
for example, in which a TH cell subpopulation-related
25 disorder situation results from a lower overall level of
target gene expression, target gene product, and/or target
gene product activity in a cell or tissue involved in such a
disorder, compounds that interact with the target gene
product can include ones which accentuate or amplify the
30 activity of the bound target gene protein. Such compounds
would bring about an effective increase in the level of
target gene activity, thus ameliorating symptoms. In
instances whereby mutations within the target gene cause
aberrant target gene proteins to be made which have a
35 deleterious effect that leads to a TH cell subpopulation-
related disorder, or, alternatively, in instances whereby
normal target gene activity is necessary for a TH cell

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subpopulation-related disorder to occur, compounds that bind
target gene protein can be identified that inhibit the
activity of the bound target gene protein. Assays for
identifying additional compounds as well as for testing the
5 effectiveness of compounds, identified by, for example,
techniques, such as those described in Section 5.8.1-5.8.3,
are discussed, below, in Section 5.8.4.

5.8.1.   IN VITRO SCREENING ASSAYS FOR COMPOUNDS THAT BIND TO A TARGET GENE PRODUCT

10
In vitro systems can be designed to identify compounds
capable of binding the target gene products of the invention.
Compounds identified can be useful, for example, in
modulating the activity of wild type and/or mutant target
15 gene products, can be useful in elaborating the biological
function of target gene products, can be utilized in screens
for identifying compounds that disrupt normal target gene
product interactions, or can in themselves disrupt such
interactions.
20   The principle of the assays used to identify compounds
that bind to the target gene product involves preparing a
reaction mixture of the target gene product and the test
compound under conditions and for a time sufficient to allow
the two components to interact and bind, thus forming a
25 complex which can be removed and/or detected in the reaction
mixture. These assays can be conducted in a variety of ways.
For example, one method to conduct such an assay would
involve anchoring target gene product or the test substance
onto a solid phase and detecting target gene product/test
30 compound complexes anchored on the solid phase at the end of
the reaction. In one embodiment of such a method, the target
gene product can be anchored onto a solid surface, and the
test compound, which is not anchored, can be labeled, either
directly or indirectly.
35   In practice, microtiter plates can conveniently be
utilized as the solid phase. The anchored component can be
immobilized by non-covalent or covalent attachments. Non-

covalent attachment can be accomplished by simply coating the
solid surface with a solution of the protein and drying.
Alternatively, an immobilized antibody, preferably a
monoclonal antibody, specific for the protein to be
5 immobilized can be used to anchor the protein to the solid
surface. The surfaces can be prepared in advance and stored.
        In order to conduct the assay, the nonimmobilized
component is added to the coated surface containing the
anchored component. After the reaction is complete,
10 unreacted components are removed (e.g., by washing) under
conditions such that any complexes formed will remain
immobilized on the solid surface. The detection of complexes
anchored on the solid surface can be accomplished in a number
of ways. Where the previously nonimmobilized component is
15 pre-labeled, the detection of label immobilized on the
surface indicates that complexes were formed. Where the
previously nonimmobilized component is not pre-labeled, an
indirect label can be used to detect complexes anchored on
the surface; e.g., using a labeled antibody specific for the
20 previously nonimmobilized component (the antibody, in turn,
can be directly labeled or indirectly labeled with a labeled
anti-Ig antibody).
        Alternatively, a reaction can be conducted in a liquid
phase, the reaction products separated from unreacted
25 components, and complexes detected; e.g., using an
immobilized antibody specific for target gene product or the
test compound to anchor any complexes formed in solution, and
a labeled antibody specific for the other component of the
possible complex to detect anchored complexes.
30   Using the 103 gene product as an example, and not by way
of limitation, techniques such as those described in this
section can be utilized to identify compounds which bind to
the 103 gene product. For example, a 103 gene product can be
contacted with a compound for a time sufficient to form a 103
35 gene product/compound complex and then such a complex can be
detected.

Alternatively, the compound can be contacted with the
103 gene product in a reaction mixture for a time sufficient
to form a 103 gene product/compound complex, and then such a
complex can be separated from the reaction mixture.

5   Among the 103 gene products which can be utilized for
such methods are, for example, rat, murine and human 103 gene
products, including, but not limited to the 103 gene products
listed in SEQ ID NOS: 39, 41, 43, 45, 47 or 48 (with or
without signal peptide sequences) or a naturally occurring
10 variant thereof.

The term "naturally occurring variant," as used herein
refers to an amino acid sequence homologous to the 103 gene
product in the same or a different species, such as, for
example, an allelic variant of the 103 gene product which
15 maps to the same chromosomal location as the nucleotide
sequences encoding the 103 gene products of SEQ ID NOS: 39,
41, 43, 45, 47 or 48, or a location syntenic to such a
location. Among the allelic variants which can be utilized
herein are allelic variant sequences encoded by a nucleotide
20 sequence that hybridizes under stringent conditions to the
complement of a nucleotide sequence encoding the 103 gene
products described above (that is SEQ ID NOS: 39, 41, 43, 45,
47 or 48, such as, for example, SEQ ID NOS: 38, 40, 42, 44,
46 or 49.

25

5.8.2.   ASSAYS FOR CELLULAR PROTEINS THAT INTERACT WITH THE
                     TARGET GENE PROTEIN
Any method suitable for detecting protein-protein
interactions can be employed for identifying novel target
30 protein-cellular or extracellular protein interactions.
These methods are outlined in Section 5.2., above, for the
identification of pathway genes, and can be utilized herein
with respect to the identification of proteins which interact
with identified target proteins.

35

5.8.3.   ASSAYS FOR COMPOUNDS THAT INTERFERE WITH TARGET GENE
          PRODUCT/CELLULAR MACROMOLECULE INTERACTION

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The target gene products of the invention can, in vivo, interact with one or more cellular or extracellular macromolecules, such as proteins. Such macromolecules can include, but are not limited to, nucleic acid molecules and
5 those proteins identified via methods such as those described, above, in Section 5.8.2. For purposes of this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partners". Compounds that disrupt such interactions can be useful in regulating the
10 activity of the target gene protein, especially mutant target gene proteins. Such compounds can include, but are not limited to molecules such as antibodies, peptides, and the like, as described, for example, in Section 5.8.1. above.

The basic principle of the assay systems used to
15 identify compounds that interfere with the interaction between the target gene product and its cellular or extracellular binding partner or partners involves preparing a reaction mixture containing the target gene product and the binding partner under conditions and for a time sufficient to
20 allow the two to interact and bind, thus forming a complex. In order to test a compound for inhibitory activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time
25 subsequent to the addition of target gene product and its cellular or extracellular binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the target gene protein and the cellular or extracellular binding
30 partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the target gene protein and the interactive binding partner. Additionally, complex formation
35 within reaction mixtures containing the test compound and normal target gene protein can also be compared to complex formation within reaction mixtures containing the test

compound and a mutant target gene protein. This comparison
can be important in those cases wherein it is desirable to
identify compounds that disrupt interactions of mutant but
not normal target gene proteins.

5   The assay for compounds that interfere with the
interaction of the target gene products and binding partners
can be conducted in a heterogeneous or homogeneous format.
Heterogeneous assays involve anchoring either the target gene
product or the binding partner onto a solid phase and
10 detecting complexes anchored on the solid phase at the end of
the reaction. In homogeneous assays, the entire reaction is
carried out in a liquid phase. In either approach, the order
of addition of reactants can be varied to obtain different
information about the compounds being tested. For example,
15 test compounds that interfere with the interaction between
the target gene products and the binding partners, e.g., by
competition, can be identified by conducting the reaction in
the presence of the test substance; i.e., by adding the test
substance to the reaction mixture prior to or simultaneously
20 with the target gene protein and interactive cellular or
extracellular binding partner. Alternatively, test compounds
that disrupt preformed complexes, e.g. compounds with higher
binding constants that displace one of the components from
the complex, can be tested by adding the test compound to the
25 reaction mixture after complexes have been formed. The
various formats are described briefly below.

In a heterogeneous assay system, either the target gene
protein or the interactive cellular or extracellular binding
partner, is anchored onto a solid surface, while the non-
30 anchored species is labeled, either directly or indirectly.
In practice, microtiter plates are conveniently utilized.
The anchored species can be immobilized by non-covalent or
covalent attachments. Non-covalent attachment can be
accomplished simply by coating the solid surface with a
35 solution of the target gene product or binding partner and
drying. Alternatively, an immobilized antibody specific for
the species to be anchored can be used to anchor the species

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to the solid surface. The surfaces can be prepared in
advance and stored.

5   In order to conduct the assay, the partner of the
immobilized species is exposed to the coated surface with or
without the test compound. After the reaction is complete,
unreacted components are removed (e.g., by washing) and any
complexes formed will remain immobilized on the solid
surface. The detection of complexes anchored on the solid
surface can be accomplished in a number of ways. Where the
10 non-immobilized species is pre-labeled, the detection of
label immobilized on the surface indicates that complexes
were formed. Where the non-immobilized species is not pre-
labeled, an indirect label can be used to detect complexes
anchored on the surface; e.g., using a labeled antibody
15 specific for the initially non-immobilized species (the
antibody, in turn, can be directly labeled or indirectly
labeled with a labeled anti-Ig antibody). Depending upon the
order of addition of reaction components, test compounds
which inhibit complex formation or which disrupt preformed
20 complexes can be detected.

Alternatively, the reaction can be conducted in a liquid
phase in the presence or absence of the test compound, the
reaction products separated from unreacted components, and
complexes detected; e.g., using an immobilized antibody
25 specific for one of the binding components to anchor any
complexes formed in solution, and a labeled antibody specific
for the other partner to detect anchored complexes. Again,
depending upon the order of addition of reactants to the
liquid phase, test compounds which inhibit complex or which
30 disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a
homogeneous assay can be used. In this approach, a preformed
complex of the target gene protein and the interactive
cellular or extracellular binding partner is prepared in
35 which either the target gene product or its binding partner
is labeled, but the signal generated by the label is quenched
due to complex formation (see, e.g., U.S. Patent

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No. 4,109,496 by Rubenstein which utilizes this approach for
immunoassays). The addition of a test substance that
competes with and displaces one of the species from the
preformed complex will result in the generation of a signal
5 above background. In this way, test substances which disrupt
target gene protein/cellular or extracellular binding partner
interaction can be identified.

In a particular embodiment, the target gene product can
be prepared for immobilization using recombinant DNA
10 techniques described in Section 5.5, above. For example, the
target gene coding region can be fused to a glutathione-S-
transferase (GST) gene using a fusion vector, such as pGEX-
5X-1, in such a manner that its binding activity is
maintained in the resulting fusion protein. The interactive
15 cellular or extracellular binding partner can be purified and
used to raise a monoclonal antibody, using methods routinely
practiced in the art and described above, in Section 5.6.
This antibody can be labeled with the radioactive isotope
^{125}I, for example, by methods routinely practiced in the art.
20 In a heterogeneous assay, e.g., the GST-target gene fusion
protein can be anchored to glutathione-agarose beads. The
interactive cellular or extracellular binding partner can
then be added in the presence or absence of the test compound
in a manner that allows interaction and binding to occur. At
25 the end of the reaction period, unbound material can be
washed away, and the labeled monoclonal antibody can be added
to the system and allowed to bind to the complexed
components. The interaction between the target gene protein
and the interactive cellular or extracellular binding partner
30 can be detected by measuring the amount of radioactivity that
remains associated with the glutathione-agarose beads. A
successful inhibition of the interaction by the test compound
will result in a decrease in measured radioactivity.

Alternatively, the GST-target gene fusion protein and
35 the interactive cellular or extracellular binding partner can
be mixed together in liquid in the absence of the solid
glutathione-agarose beads. The test compound can be added

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either during or after the species are allowed to interact.
This mixture can then be added to the glutathione-agarose
beads and unbound material is washed away. Again the extent
of inhibition of the target gene product/binding partner

5 interaction can be detected by adding the labeled antibody
and measuring the radioactivity associated with the beads.

In another embodiment of the invention, these same
techniques can be employed using peptide fragments that
correspond to the binding domains of the target gene product

10 and/or the interactive cellular or extracellular binding
partner (in cases where the binding partner is a protein), in
place of one or both of the full length proteins. Any number
of methods routinely practiced in the art can be used to
identify and isolate the binding sites. These methods

15 include, but are not limited to, mutagenesis of the gene
encoding one of the proteins and screening for disruption of
binding in a co-immunoprecipitation assay. Compensating
mutations in the gene encoding the second species in the
complex can then be selected. Sequence analysis of the genes

20 encoding the respective proteins will reveal the mutations
that correspond to the region of the protein involved in
interactive binding. Alternatively, one protein can be
anchored to a solid surface using methods described in this
Section above, and allowed to interact with and bind to its

25 labeled binding partner, which has been treated with a
proteolytic enzyme, such as trypsin. After washing, a short,
labeled peptide comprising the binding domain can remain
associated with the solid material, which can be isolated and
identified by amino acid sequencing. Also, once the gene

30 coding for the cellular or extracellular binding partner is
obtained, short gene segments can be engineered to express
peptide fragments of the protein, which can then be tested
for binding activity and purified or synthesized.

For example, and not by way of limitation, a target gene

35 product can be anchored to a solid material as described,
above, in this Section, by making a GST-target gene fusion
protein and allowing it to bind to glutathione agarose beads.

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The interactive cellular or extracellular binding partner can
be labeled with a radioactive isotope, such as ^{35}S, and
cleaved with a proteolytic enzyme such as trypsin. Cleavage
products can then be added to the anchored GST-target gene
5 fusion protein and allowed to bind. After washing away
unbound peptides, labeled bound material, representing the
cellular or extracellular binding partner binding domain, can
be eluted, purified, and analyzed for amino acid sequence by
well known methods. Peptides so identified can be produced
10 synthetically or fused to appropriate facilitative proteins
using well known recombinant DNA technology.

5.8.4. ASSAYS FOR AMELIORATION OF IMMUNE DISORDER SYMPTOMS AND/OR THE MODULATION OF TARGET GENE PRODUCT FUNCTION

15   Any of the binding compounds, including but not limited
to, compounds such as those identified in the foregoing assay
systems, can be tested for the ability to ameliorate symptoms
of immune disorders e.g., TH cell subpopulation-related
disorders. Cell-based and animal model-based assays for the
20 identification of compounds exhibiting such an ability to
ameliorate immune disorder symptoms are described below.
Further, cell-based assays for the identification of
compounds which modulate target gene product function, in
instances where the target gene product is a receptor having
25 a seven transmembrane domain sequence, such as, for example,
that of the 10 gene product, are described, below, in Section
5.8.4.1.

        First, cell-based systems such as those described,
above, in Section 5.7.2, can be used to identify compounds
30 which can act to ameliorate TH cell subpopulation-related
disorder symptoms. For example, such cell systems can be
exposed to a compound, suspected of exhibiting an ability to
ameliorate the disorder symptoms, at a sufficient
concentration and for a time sufficient to elicit such an
35 amelioration in the exposed cells. After exposure, the cells
are examined to determine whether one or more of the TH cell
subpopulation-related disorder-like cellular phenotypes has

been altered to resemble a phenotype more likely to produce a
lower incidence or severity of disorder symptoms. Additional
cell-based assays are discussed, below, in Section 5.8.4.1.

　　　　Taking the TH cell subpopulation-related disorder
5 asthma, which is, specifically, a TH2-like-related disorder,
any TH2 or TH2-like cell system can be utilized. Upon
exposure to such cell systems, compounds can be assayed for
their ability to modulate the TH2-like phenotype of such
cells, such that the cells exhibit loss of a TH2-like
10 phenotype. Compounds with such TH2 modulatory capability
represent ones which can potentially exhibit the ability to
ameliorate asthma-related symptoms in vivo. The Example
presented in Section 12, below, describes the successful
utilization of a 103 gene product/Ig fusion protein, as well
15 as the successful use of a monoclonal antibody directed
against the extracellular domain of the 103 gene product to
ameliorate symptoms of asthma in an accepted animal model of
asthma.

　　　　In addition, animal-based systems, such as those
20 described, above, in Section 5.7.1, can be used to identify
compounds capable of ameliorating TH cell subpopulation-
related disorder-like symptoms. Such animal models can be
used as test substrates for the identification of drugs,
pharmaceuticals, therapies, and interventions which can be
25 effective in treating such disorders. For example, animal
models can be exposed to a compound, suspected of exhibiting
an ability to ameliorate TH cell subpopulation-related
disorder symptoms, at a sufficient concentration and for a
time sufficient to elicit such an amelioration of the
30 symptoms in the exposed animals. The response of the animals
to the exposure, and thus the efficacy of the compound in
question, can be monitored by assessing the reversal of
disorders associated with TH cell subpopulation-related
disorders of interest. With regard to intervention, any
35 treatments which reverse any aspect of TH cell subpopulation-
related disorder-like symptoms should be considered as
candidates for corresponding human TH cell subpopulation-

related disorder therapeutic intervention. Dosages of test
agents can be determined by deriving dose-response curves, as
discussed in Section 5.11, below.

        Gene expression patterns can be utilized in conjunction
5 with either cell-based or animal-based systems, to assess the
ability of a compound to ameliorate TH cell subpopulation-
related disorder-like symptoms. For example, the expression
pattern of one or more fingerprint genes can form part of a
fingerprint profile which can be then be used in such an
10 assessment. Fingerprint profiles are described, below, in
Section 5.12. Fingerprint profiles can be characterized for
known states, either TH cell subpopulation-related disorder
states, or normal TH cell differentiative states, within the
cell- and/or animal-based model systems.
15

    5.9.   METHODS FOR THE IDENTIFICATION OF COMPOUNDS WHICH
            MODULATE TARGET GENE PRODUCT FUNCTION
        In this Section, methods are described for the
identification of compounds which act as either agonists or
20 antagonists of receptor target gene products. The 10 gene
product (FIG. 9; SEQ ID NO:9) is an example of a seven
transmembrane domain target gene product. For ease of
explanation, and not by way of limitation, therefore, the 10
gene product will be used to illustrate the methods described
25 in this Section.
        The compounds tested may be, for example, compounds such
as those identified via the assays described, above, in
Sections 5.8.1 to 5.8.3. Such compounds may include, but are
not limited to peptides such as, for example, soluble
30 peptides, including, but not limited to, Ig-tailed fusion
peptides, comprising extracellular portions of target gene
product transmembrane receptors, and members of random
peptide libraries (see, e.g., Lam, K.S. et al., 1991, Nature
354:82-84; Houghten, R. et al., 1991, Nature 354:84-86) made
35 of D-and/or L-configuration amino acids, phosphopeptides
(including but not limited to members of random or partially
degenerate, directed phosphopeptide libraries; see, e.g.,

Songyang, Z. et al., 1993, Cell 72:767-778), antibodies
(including, but not limited to polyclonal, monoclonal,
humanized, anti-idiotypic, chimeric or single chain
antibodies, and FAb, F(ab')$_2$ and FAb expression library
5 fragments, and epitope-binding fragments thereof), and small
organic or inorganic molecules.

    The assays described herein are functional assays which
identify compounds that affect the receptor target gene's
activity by affecting the level of intracellular calcium
10 release within cells expressing such seven transmembrane
domain receptor target protein (e.g., the 10 gene product).
Intracellular calcium release is measured because such seven
transmembrane domain receptors tend to be G protein-coupled
receptors and because activation of these receptors leads to
15 a G protein-mediated intracellular calcium release.
Modulation (i.e., agonization or antagonization) of the
receptor target gene product function, then, would result in
a difference in intracellular calcium levels.

    The assays comprise contacting a seven transmembrane
20 domain receptor target gene-expressing cell with a test
compound and measuring the level of intracellular calcium.
Those compounds which produce an intracellular calcium
profile which differs from that which the cell would exhibit
in the absence of the compound represent either agonists or
25 antagonists. An agonist compound would cause an increase in
intracellular calcium levels relative to control cells while
an antagonist would result in a decrease in intracellular
calcium levels relative to control cells.

    While any cell expressing a seven transmembrane receptor
30 target gene product may be used herein, it is preferred that
cells be used whose intracellular calcium levels may readily
measured. Xenopus oocytes, due to their large size, are
among such preferred cells because they can easily be
injected with intracellular calcium reporter compounds.
35 Additionally, myeloma cells may be utilized. Such reporter
compounds include, but are not limited to, calcium-binding
agents such as the well known FURA-2 and INDO-2. FURA-

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2/calcium complexes and INDO-2/calcium complexes fluoresce,
making possible the measurement of differences in
intracellular calcium levels.

For the purposes of the assays described herein, the
5 Xenopus oocytes should be transfected with nucleotide
sequences encoding the target protein of interest (e.g., the
10 gene product). The cells can be transfected and express
the sequence of interest via techniques which are well known
to those of skill in the art and which may include, for
10 example, techniques such as those described, above, in
Section 5.5. Xenopus oocytes can be injected with RNA
encoding the target gene product of interest such that the
injected oocytes will express the gene product.

The assays described in this Section may, first, be used
15 to identify compounds which act as agonists of the target
gene product of interest, e.g., the 10 gene product.
"Agonist", as used herein, refers to a compound which
modulates target gene product activity by increasing the
target gene product's activity, as evaluated by the
20 compound's ability to bring about an increase in calcium
influx, leading to an increase intracellular calcium levels.
Among such agonists can be, for example, the natural ligand
for the receptor target gene product, e.g., the natural
ligand for the 10 gene product.

25   Agonists identified via such assays may act as useful
therapeutic agents for the amelioration of a wide range of T
cell-related disorders, including, for example, TH cell
subpopulation-related disorders, in instances whereby such
disorders are caused by a reduced or absent level of target
30 gene product activity. Any of the agonist compounds
identified herein can be used, for example, as part of the
treatment methods described in Section 5.9.2, below.
Further, such agonists can be used to identify antagonists of
the receptor target gene product of interest, e.g., as
35 described, below.

"Antagonist", as used herein, refers to a compound which
modulates target gene product activity by decreasing the

target gene product's activity, as evaluated by the
compound's ability to bring about a decrease in calcium
influx. Antagonists identified via such assays may act as
useful therapeutic agents for the amelioration of a wide
5 range of T cell-related disorders, including, for example, TH
cell subpopulation-related disorders, in instances whereby
the disorder is caused by an increased or inappropriate level
of target gene product activity.

An antagonist screen may be performed utilizing target
10 gene product-expressing cells as described, above, and which
include, but are not limited to, such cells as 10 gene-
expressing cells, for example, 10 gene-expressing Xenopus
oocytes. In those instances whereby the T cell-related
disorder is caused by a mutant target gene product, the cells
15 utilized in the antagonist assay can be cells which express
the mutant receptor target gene product involved in causing
the T cell-related disorder.

To conduct an antagonist screen, a target gene-
expressing cell is contacted with 1) an agonist of the target
20 gene product and 2) a test compound for a given period of
time. The level of intracellular calcium is then measured in
the cells and in cells which have been contacted with agonist
alone. A test compound is considered to be an antagonist if
the level of intracellular calcium release in the presence of
25 the test compound is lower than the level of intracellular
calcium release in the absence of the test compound.

Any of the antagonist compounds identified herein can be
used, for example, as part of the treatment methods
described, below, in Section 5.9.1.
30   Among the potential antagonist compounds of the seven
transmembrane domain receptor target gene products described
herein are peptides which contain one or more of the receptor
target gene product's extracellular domains, preferably those
domains are domains which are responsible for ligand-binding
35 such that the peptides act to compete with the endogenous
receptor for ligand. In the case of the 10 gene product, for
example, such extracellular domains include from

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approximately 10 gene product amino acid residue 1 to 19, amino acid residue 74 to 87, amino acid residue 153-187 and amino acid residue 254 to 272. Such extracellular domain antagonist compounds may comprise soluble Ig-tailed fusion
5 proteins which may be produced by utilizing techniques such as those described, above, in Section 5.5. Additionally, antibodies directed against the extracellular portion of the 10 gene product may reduce 10 gene product function by, for example, blocking ligand binding.
10

5.10. COMPOUNDS AND METHODS FOR TREATMENT OF IMMUNE DISORDERS AND FOR MODULATION OF TH CELL RESPONSIVENESS

Described below are methods and compositions which can be used to ameliorate immune disorder symptoms via, for
15 example, a modulation of the TH cell subpopulation of interest. Such modulation can be of a positive or negative nature, depending on the specific situation involved, but each modulatory event yields a net result in which symptoms of the immune disorder are ameliorated. Further, described
20 below are methods for the modulation of TH cell responsiveness to antigen.

"Negative modulation", as used herein, refers to a reduction in the level and/or activity of target gene product relative to the level and/or activity of the target gene
25 product in the absence of the modulatory treatment. Alternatively, the term, as used herein, refers to a depletion of the T cell subpopulation (e.g., via a reduction in the number of cells belonging to the TH cell subpopulation) relative to the number present in the absence
30 of the modulatory treatment. "Depletion," as used herein, is as defined, above, in Section 3.

"Positive modulation", as used herein, refers to an increase in the level and/or activity of target gene product relative to the level and/or activity of the gene product in
35 the absence of the modulatory treatment. Alternatively, the term, as used herein, refers to a stimulation of the T cell subpopulation (e.g., via an increase in the number of cells

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belonging to the TH cell subpopulation), relative to the
number present in the absence of the modulatory treatment.
"Stimulation," as used herein, is as defined, above, in
Section 3.

5   It is possible that a TH cell subpopulation-related
disorder or other immune disorder, can occur as a result of
normal target gene activity during the course of, for
example, exposure to a certain antigen which elicits an
immune response that leads to the development of the
10 disorder. For example, the TH2-like-related disorders,
asthma and allergy, are likely candidates of disorders having
such a mechanism. Additionally, a disorder can be brought
about, at least in part, by an abnormally high level of
target gene product, or by the presence of a target gene
15 product exhibiting an abnormal activity. As such, a
technique which elicits a negative modulatory effect, _i.e._,
brings about a reduction in the level and/or activity of
target gene product, or alternatively, brings about a
depletion of the TH cell subpopulation (_e.g._, via a physical
20 reduction in the number of cells belonging to the TH cell
subpopulation), would effect an amelioration of TH cell
subpopulation-related disorder symptoms in either of the
above scenarios.

Negative modulatory techniques for the reduction of
25 target gene expression levels or target gene product activity
levels, (either normal or abnormal), and for the reduction in
the number of specific TH cell subpopulation cells are
discussed in Section 5.9.1, below.

Alternatively, it is possible that a TH cell
30 subpopulation-related disorder or other immune disorders can
be brought about, at least in part, by the absence or
reduction of the level of target gene expression, a reduction
in the level of a target gene product's activity, or a
reduction in the overall number of cells belonging to a
35 specific TH cell subpopulation. As such, a technique which
elicits a positive modulatory effect, _i.e._, brings about an
increase in the level of target gene expression and/or the

activity of such gene products, or, alternatively, a
stimulation of the TH cell subpopulation (e.g., via a
physical increase in the number of cells belonging to a TH
cell subpopulation), would effect an amelioration of immune
5 disorder symptoms.

For example, a reduction in the overall number of TH1-
like cells relative to TH2-like cells within a HIV-infected
individual can correlate with the progression to AIDS
(Clerci, M. et al., 1993, J. Clin. Invest. 91:759; Clerci, M.
10 et al., 1993, Science 262:1721; Maggi, E. et al., 1994,
Science 265:244). A treatment capable of increasing the
number of TH1-like cells relative to TH2-like cells within an
HIV-infected individual may, therefore, serve to prevent or
slow the progression to disease.

15   Positive modulatory techniques for increasing target
gene expression levels or target gene product activity
levels, and for increasing the level of specific TH cell
subpopulation cells are discussed, below, in Section 5.9.2.

Among the immune disorders whose symptoms can be
20 ameliorated are TH1 or TH1-like related immune disorders and
TH2 or TH2-like related immune disorders. Examples of TH1 or
TH1-like related disorders include chronic inflammatory
diseases and disorders, such as Crohn's disease, reactive
arthritis, including Lyme disease, insulin-dependent
25 diabetes, organ-specific autoimmunity, including multiple
sclerosis, Hashimoto's thyroiditis and Grave's disease,
contact dermatitis, psoriasis, graft rejection, graft versus
host disease and sarcoidosis. Examples of TH2 or TH2-like
related disorders include atopic conditions, such as asthma
30 and allergy, including allergic rhinitis, gastrointestinal
allergies, including food allergies, eosinophilia,
conjunctivitis, glomerular nephritis, certain pathogen
susceptibilities such as helminthic (e.g., leishmaniasis) and
certain viral infections, including HIV, and bacterial
35 infections, including tuberculosis and lepromatous leprosy.

The methods described herein can additionally be
utilized the modulate the level of responsiveness, for

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example, responsiveness to antigen, of a TH cell
subpopulation. Such methods are important in that many
immune disorders involve inappropriate rather than
insufficient immune responses. For example, disorders such
5 as atopic, IgE-mediated allergic conditions, including
asthma, pathogen susceptibilities and chronic inflammatory
disease, involve strong but counterproductive TH2-mediated
immune responses. Further, inappropriate TH1-mediated immune
responses to self-antigens is central to the development of
10 such disorders as multiple sclerosis, psoriasis, insulin
dependent diabetes, Hashimoto's thyroiditis and Crohn's
disease.

Methods for modulating TH cell responsiveness can
comprise, for example, contacting a compound to a TH cell so
15 that the responsiveness of the T helper cell is modulated
relative to the responsiveness of the T helper cell in the
absence of the compound. The modulation can increase or
decrease the responsiveness of the TH cell. Any of the
techniques described, below, in Sections 5.9.1-5.9.3.2 can be
20 utilized to effect an appropriate modulation of TH cell
responsiveness.

Still further, the methods and techniques described
herein can also be used for treating, ameliorating, or
modulating symptoms associated with mast cell-related
25 processes or disorders or certain ischemia disorders or
injuries. For example, techniques which increase the
expression or activity of certain gene products of the
invention whose activity is involved in the repair of
ischemic injury or damage, particularly techniques which
30 increase the expression or activity of the 200 gene product
of this invention, can be used to treat tissue or organ
damage produced by an ischemic disorder or injury.
Alternatively, techniques or methods which inhibit the
expression or activity of target gene products of the
35 invention can be used to block or inhibit the repair of
ischemic tissue or organs. Such techniques are useful, for
example, to treat ischemic or infarcated tissue, such as a

cancerous tumor, to increase damage or injury to such tissue, e.g., during treatment such as chemotherapy.

Among the ischemic disorders or injuries whose symptoms can be ameliorated are ischemic renal disease and myocardial
5 ischemia, such as angina pectoris, as well as ischemic injuries to other tissues including, but by no means limited to, the brain (as in a stroke), spleen, intestine, lung, and testes. Such techniques can also be used to treat, or to enhance ischemic injuries to tumors, including tumors of the
10 ovary or uterus.

The methods described herein can additionally be used to treat or prevent ischemic damage or injury to transplanted · organs, such as transplanted kidneys, lungs, hearts, livers, and pancrease, or grafts, such as skin grafts. Such
15 techniques are important in that some ischemic damage to transplanted organs typically occurs during transplant from donor to host, when oxygen perfusion to tissue of the transplanted organ is severely reduced.

Among the genes and gene products identified and present
20 in the present invention, the 200 gene is demonstrated in Section 13, herein, to play a critical role in the resolution (i.e., the repair) of injury following ischemia reperfusion. The 200 gene and its products can, therefore, be utilized in the treatment of ischemic disorders and injuries. For
25 example, a gene 200 product, or functional portions thereof, can be utilized either directly or indirectly to stimulate or increase the repair of injury to tissue or organs resulting from an ischemic injury or disorder.

30
5.10.1. NEGATIVE MODULATORY TECHNIQUES

As discussed, above, successful treatment of certain immune disorders can be brought about by techniques which serve to inhibit the expression or activity of target gene
35 products, or which, alternatively, serve to reduce the overall number of cells belonging to a specific TH cell subpopulation. Further, as also described above, techniques

which serve to inhibit the expression or activity of target
gene products of the present invention, including the 200
gene product, can be used to inhibit the repair or recovery
of certain ischemic tissue.

5   For example, compounds such as those identified through
assays described, above, in Section 5.8, which exhibit
negative modulatory activity, can be used in accordance with
the invention to ameliorate certain TH cell subpopulation-
related disorder symptoms, or, alternatively, to inhibit the
10 repair or recovery of ischemic tissue. As discussed in
Section 5.8, above, such molecules can include, but are not
limited to peptides (such as, for example, peptides
representing soluble extracellular portions of target gene
product transmembrane receptors), phosphopeptides, small
15 organic or inorganic molecules, or antibodies (including, for
example, polyclonal, monoclonal, humanized, anti-idiotypic,
chimeric or single chain antibodies, and FAb, $F(ab')_2$ and FAb
expression library fragments, and epitope-binding fragments
thereof). In one embodiment, for example, antibodies
20 directed against a 103 gene product, preferably an
extracellular or extracellular portion of a 103 gene product,
can be utilized. Techniques for the determination of
effective doses and administration of such compounds are
described, below, in Section 5.11.

25   Further, antisense and ribozyme molecules which inhibit
expression of the target gene can also be used in accordance
with the invention to reduce the level of target gene
expression, thus effectively reducing the level of target
gene activity. Still further, triple helix molecules can be
30 utilized in reducing the level of target gene activity. Such
techniques are described, below.

Additionally, techniques for the depletion of specific
TH cell subpopulations are discussed, below, in Section
5.10.3.2. Such techniques can take advantage of, for
35 example, novel cell surface markers which are specific to the
TH cell subpopulation to be depleted, and can include in vivo
or in vitro targeted destruction, or, alternatively,

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selective purification away, of the TH cell subpopulation of
interest.

Among the TH cell subpopulation-related sequences
identified by the methods described by the present invention
5 is a gene designated herein as the 103 gene, as discussed in
the Example presented in Section 7, below. The 103 gene is
demonstrated herein to represent a TH2-specific gene in that
103 gene expression is found to be absent TH1 cells as well
as all other tissues tested. Further, at least one of the
10 proteins produced by the 103 gene is a transmembrane protein.

The 103 gene and its products can, therefore, be
utilized in the treatment of TH2 cell subpopulation-related
disorders. For example, a 103 gene product or portions
thereof can be utilized, either directly or indirectly, to
15 ameliorate conditions involving inappropriate IgE immune
responses, including, but not limited to the symptoms which
accompany atopic conditions such as allergy and/or asthma.
IgE-type antibodies are produced by stimulated B cells which
require, at least in part, IL-4 produced by the TH2 cell
20 subpopulation. Therefore, any treatment, including, for
example, the use of a gene 103 product or portion thereof,
which reduces the effective concentration of secreted IL-4,
e.g., by reducing the number or activity of TH2 cells, can
bring about a reduction in the level of circulating IgE,
25 leading, in turn, to the amelioration of the conditions
stemming from an inappropriate IgE immune response.

There exist a variety of ways in which the TH2 specific
103 gene products can be used to effect such a reduction in
the activity and/or effective concentration of TH2 cells.
30   For example, natural ligands, derivatives of natural
ligands and antibodies which bind to the 103 gene product can
be utilized to reduce the number of TH2 cells present by
either physically separating such cells away from other cells
in a population, thereby deleting the TH2 cell subpopulation,
35 or, alternatively, by targeting the specific destruction of
TH2 cells. Such techniques are discussed, below, in Section

5.9.3.   Further, such compounds can be used to inhibit the
proliferation of TH2 cells.

Additionally, compounds such as 103 gene sequences or
gene products can be utilized to reduce the level of TH2 cell
5 activity, cause a reduction in IL-4 production, and,
ultimately, bring about the amelioration of IgE related
disorders.

For example, compounds can be administered which compete
with endogenous ligand for the 103 gene product. The
10 resulting reduction in the amount of ligand-bound 103 gene
transmembrane protein will modulate TH2 cellular activity.
Compounds which can be particularly useful for this purpose
include, for example, soluble proteins or peptides, such as
peptides comprising the extracellular domain, or portions
15 and/or analogs thereof, of the gene 103 product, including,
for example, soluble fusion proteins such as Ig-tailed fusion
proteins. (For a discussion of the production of Ig-tailed
fusion proteins see, for example, U.S. Patent No. 5,
116,964.)
20   Production of a 103 gene product/Ig fusion is described
in Section 10, below. Further, use of a 103 gene product/Ig
fusion to successfully ameliorate symptoms in an accepted
animal model for asthma is described in Section 12, below.

The novel 200 gene, which encodes a receptor target gene
25 product that is a member of the Ig superfamily, exhibits a
TH1-specific pattern of gene expression. The 200 gene,
especially the human 200 gene, and its products can,
therefore, be utilized in the treatment of TH1 cell
subpopulation-related disorders such as, for example, chronic
30 inflammatory diseases, psoriasis, graft rejection and graft
versus host disease.

The treatment of such disorder may require a reduction
in the activity and/or effective concentration of the TH1
cell subpopulation involved in the disorder of interest. As
35 such, a number of methods exist whereby the TH1 specific 200
gene products can be used to effect such a reduction in the
activity and/or effective concentration of TH1 cells.

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For example, natural ligands, derivatives of natural
ligands and antibodies which bind to the 200 gene product can
be utilized to reduce the number of TH1 cells present by
either physically separating such cells away from other cells
5 in a population, thereby deleting the TH1 cell subpopulation,
or, alternatively, by targeting the specific destruction of
TH1 cells. Such techniques are discussed, below, in Section
5.10.3. Further, such compounds can be used to inhibit the
proliferation of TH1 cells.

10   Additionally, compounds can be administered which
compete with endogenous ligand for the 200 gene product.
Such compounds would bind to and "neutralize" circulating
ligand. The resulting reduction in the amount of ligand-
bound 200 gene transmembrane protein will modulate TH1
15 cellular activity. Further, reduction in the amount of
ligand bound 200 gene transmembrane protein will also inhibit
the resolution and repair of ischemic tissue.

Compounds which can be particularly useful for this
purpose include, for example, soluble proteins or peptides,
20 such as peptides comprising the extracellular domain, or
portions and/or analogs thereof, of the gene 200 product,
including, for example, soluble fusion proteins such as Ig-
tailed fusion proteins or antibodies. (For a discussion of
the production of Ig-tailed fusion proteins see, for example,
25 U.S. Patent No. 5, 116,964.)

To this end, peptides corresponding to the ECD of the
200 gene product, soluble deletion mutants of 200 gene
product, or either of these 200 gene product domains or
mutants fused to another polypeptide (e.g., an IgFc
30 polypeptide) can be utilized. Alternatively, anti-idiotypic
antibodies or Fab fragments of antiidiotypic antibodies that
mimic the 200 gene product ECD and neutralize 200 gene
product ligand can be used. Such 200 gene product peptides,
proteins, fusion proteins, anti-idiotypic antibodies or Fabs
35 are administered to a subject in amounts sufficient to
neutralize the gene product and thereby effectuate an

amelioration of a T cell subpopulation-related disorder, or
an inhibition of repair of ischemic tissues.

200 gene product peptides corresponding to the ECD
having the amino acid sequence shown in FIG. 17 (SEQ ID
5 NO:10) from about amino acid residue number 21 to about 192
can be used. Human 200 gene product peptides corresponding
to the ECD having the amino acid sequence shown in FIG. 24
(SEQ ID NO:24) from approximately amino acid reside number 21
to about 200. Mutants in which all or part of the
10 hydrophobic anchor sequence (e.g., about amino acid residue
number 193 to 214 in FIG. 17, or about 201 to about 224 in
FIG. 24) is deleted could also be used. Fusion of these
peptides to an IgFc polypeptide should not only increase the
stability of the preparation, but will increase the half-life
15 and activity of the fusion protein _in vivo_. The Fc region of
the Ig portion of the fusion protein may be further modified
to reduce immunoglobulin effector function. For example,
nucleotide sequences encoding the fusion protein may be
modified to encode fusion proteins which replace cysteine
20 residues in the hinge region with serine residues and/or
amino acids within the CH2 domain believed to be required for
IgC binding to FC receptors and complement activation.

In an alternative embodiment for neutralizing
circulating 200 gene product ligand, cells that are
25 genetically engineered to express such soluble or secreted
forms of 200 gene product may be administered to a patient,
whereupon they will serve as "bioreactors" _in vivo_ to provide
a continuous supply of the 200 gene product ligand
neutralizing protein. Such cells may be obtained from the
30 patient or an MHC compatible donor and can include, but are
not limited to fibroblasts, blood cells (e.g., lymphocytes),
adipocytes, muscle cells, endothelial cells etc. The cells
are genetically engineered _in vitro_ using recombinant DNA
techniques to introduce the coding sequence for the 200 gene
35 product peptide, or 2C0 gene product fusion proteins
(discussed above) into the cells, e.g., by transduction
(using viral vectors, and preferably vectors that integrate

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the transgene into the cell genome) or transfection
procedures, including but not limited to the use of plasmids,
cosmids, YACs, electroporation, liposomes, etc. The 200 gene
product coding sequence can be placed under the control of a

5 strong constitutive or inducible promoter or
promoter/enhancer to achieve expression and secretion of the
200 gene peptide or fusion protein. The engineered cells
which express and secrete the desired 200 gene product can be
introduced into the patient systemically, *e.g.*, in the

10 circulation, or intrapertioneally. Alternatively, the cells
can be incorporated into a matrix and implanted in the body,
e.g., genetically engineered fibroblasts can be implanted as
part of a skin graft; genetically engineered endothelial
cells can be implanted as part of a vascular graft. (See,

15 for example, Anderson et al. U.S. Patent No. 5,399,349; and
Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is
incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous
cells, they can be administered using well known techniques

20 which prevent the development of a host immune response
against the introduced cells. For example, the cells may be
introduced in an encapsulated form which, while allowing for
an exchange of components with the immediate extracellular
environment, does not allow the introduced cells to be

25 recognized by the host immune system.

It is to be understood that, while such approaches and
techniques are described, for sake of clarity, using the 200
gene product as an example, they may be applied to any of the
target and/or pathway gene products having such receptor-type

30 structures.

The 10 gene product is identified herein as a receptor
target gene product having a seven transmembrane domain
sequence motif. Further, the 10 gene is shown to exhibit a
TH inducible pattern of expression, meaning that 10 gene

35 expression increases in both TH1 and TH2 cell subpopulations
in response to stimulation and can important to T cell
responses in general. The 10 gene and its products can,

therefore, be utilized in the treatment of a wide T cell-
related disorders. Techniques such as those described,
above, for the 103 and the 200 genes and gene products can
also be utilized for the amelioration of disorders in which
5 10 gene expression is involved.

Modulatory Antisense, Ribozyme, and Triple Helix Approaches:

Among the compounds which can exhibit the ability to
10 ameliorate TH cell subpopulation-related disorder symptoms
are antisense, ribozyme, and triple helix molecules. Such
molecules can be designed to reduce or inhibit either wild
type, or if appropriate, mutant target gene activity.
Techniques for the production and use of such molecules are
15 well known to those of skill in the art.

Antisense approaches involve the design of
oligonucleotides (either DNA or RNA) that are complementary
to target or pathway gene mRNA. The antisense
oligonucleotides will bind to the complementary target or
20 pathway gene mRNA transcripts and prevent translation.
Absolute complementarity, although preferred, is not
required. A sequence "complementary" to a portion of an RNA,
as referred to herein, means a sequence having sufficient
complementarity to be able to hybridize with the RNA, forming
25 a stable duplex; in the case of double-stranded antisense
nucleic acids, a single strand of the duplex DNA may thus be
tested, or triplex formation may be assayed. The ability to
hybridize will depend on both the degree of complementarity
and the length of the antisense nucleic acid. Generally, the
30 longer the hybridizing nucleic acid, the more base mismatches
with an RNA it may contain and still form a stable duplex (or
triplex, as the case may be). One skilled in the art can
ascertain a tolerable degree of mismatch by use of standard
procedures to determine the melting point of the hybridized
35 complex.

Oligonucleotides that are complementary to the 5' end of
the message, e.g., the 5' untranslated sequence up to and

including the AUG initiation codon, should work most
efficiently at inhibiting translation. However, sequences
complementary to the 3' untranslated sequences of mRNAs have
recently been shown to be effective at inhibiting translation
5 of mRNAs as well. See generally, Wagner, R., 1994, Nature
372:333-335. Thus, oligonucleotides complementary to either
the 5'- or 3'- non-translated, non-coding regions of target
or pathway genes, as shown, for example, in Fig. 9, 17, 22,
23 and 24, could be used in an antisense approach to inhibit
10 translation of endogenous target or pathway gene mRNA.
Oligonucleotides complementary to the 5' untranslated region
of the mRNA should include the complement of the AUG start
codon. Antisense oligonucleotides complementary to mRNA
coding regions are less efficient inhibitors of translation
15 but could be used in accordance with the invention. Whether
designed to hybridize to the 5'-, 3'- or coding region of
target or pathway gene mRNA, antisense nucleic acids should
be at least six nucleotides in length, and are preferably
oligonucleotides ranging from 6 to about 50 nucleotides in
20 length. In specific aspects the oligonucleotide is at least
10 nucleotides, at least 17 nucleotides, at least 25
nucleotides or at least 50 nucleotides.

Regardless of the choice of target sequence, it is
preferred that *in vitro* studies are first performed to
25 quantitate the ability of the antisense oligonucleotide to
inhibit gene expression. It is preferred that these studies
utilize controls that distinguish between antisense gene
inhibition and nonspecific biological effects of
oligonucleotides. It is also preferred that these studies
30 compare levels of the target RNA or protein with that of an
internal control RNA or protein. Additionally, it is
envisioned that results obtained using the antisense
oligonucleotide are compared with those obtained using a
control oligonucleotide. It is preferred that the control
35 oligonucleotide is of approximately the same length as the
test oligonucleotide and that the nucleotide sequence of the
oligonucleotide differs from the antisense sequence no more

than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-
5 stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host
10 cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain
15 barrier (see, e.g., PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents. (See, e.g., Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be
20 conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group
25 including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-
30 galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil,
35 beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil,

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queosine, 2-thiocytosine, 5-methyl-2-thiouracil,
2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-
5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v),
5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl)
5 uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least
one modified sugar moiety selected from the group including
but not limited to arabinose, 2-fluoroarabinose, xylulose,
and hexose.

10   In yet another embodiment, the antisense oligonucleotide
comprises at least one modified phosphate backbone selected
from the group consisting of a phosphorothioate, a
phosphorodithioate, a phosphoramidothioate, a
phosphoramidate, a phosphordiamidate, a methylphosphonate, an
15 alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide
is an α-anomeric oligonucleotide. An α-anomeric
oligonucleotide forms specific double-stranded hybrids with
complementary RNA in which, contrary to the usual β-units,
20 the strands run parallel to each other (Gautier et al., 1987,
Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-
0-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res.
15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al.,
1987, FEBS Lett. 215:327-330).

25   Oligonucleotides of the invention may be synthesized by
standard methods known in the art, e.g. by use of an
automated DNA synthesizer (such as are commercially available
from Biosearch, Applied Biosystems, etc.). As examples,
phosphorothioate oligonucleotides may be synthesized by the
30 method of Stein et al. (1988, Nucl. Acids Res. 16:3209),
methylphosphonate oligonucleotides can be prepared by use of
controlled pore glass polymer supports (Sarin et al., 1988,
Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

The antisense molecules should be delivered to cells
35 which express the target or pathway gene in vivo. A number
of methods have been developed for delivering antisense DNA
or RNA to cells; e.g., antisense molecules can be injected

directly into the tissue site, or modified antisense
molecules, designed to target the desired cells (e.g.,
antisense linked to peptides or antibodies that specifically
bind receptors or antigens expressed on the target cell
5 surface) can be administered systemically.

However, it is often difficult to achieve intracellular
concentrations of the antisense sufficient to suppress
translation of endogenous mRNAs. Therefore a preferred
approach utilizes a recombinant DNA construct in which the
10 antisense oligonucleotide is placed under the control of a
strong pol III or pol II promoter. The use of such a
construct to transfect target cells in the patient will
result in the transcription of sufficient amounts of single
stranded RNAs that will form complementary base pairs with
15 the endogenous target or pathway gene transcripts and thereby
prevent translation of the target or pathway gene mRNA. For
example, a vector can be introduced in vivo such that it is
taken up by a cell and directs the transcription of an
antisense RNA. Such a vector can remain episomal or become
20 chromosomally integrated, as long as it can be transcribed to
produce the desired antisense RNA. Such vectors can be
constructed by recombinant DNA technology methods standard in
the art. Vectors can be plasmid, viral, or others known in
the art, used for replication and expression in mammalian
25 cells. Expression of the sequence encoding the antisense RNA
can be by any promoter known in the art to act in mammalian,
preferably human cells. Such promoters can be inducible or
constitutive. Such promoters include but are not limited to:
the SV40 early promoter region (Bernoist and Chambon, 1981,
30 Nature 290:304-310), the promoter contained in the 3' long
terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980,
Cell 22:787-797), the herpes thymidine kinase promoter
(Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-
1445), the regulatory sequences of the metallothionein gene
35 (Brinster et al., 1982, Nature 296:39-42), etc. Any type of
plasmid, cosmid, YAC or viral vector can be used to prepare
the recombinant DNA construct which can be introduced

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directly into the tissue site. Alternatively, viral vectors
can be used which selectively infect the desired tissue.

Ribozymes are enzymatic RNA molecules capable of
catalyzing the specific cleavage of RNA (For a review see,
5 for example Rossi, J., 1994, Current Biology 4:469-471). The
mechanism of ribozyme action involves sequence specific
hybridization of the ribozyme molecule to complementary
target RNA, followed by a endonucleolytic cleavage. The
composition of ribozyme molecules must include one or more
10 sequences complementary to the target gene mRNA, and must
include the well known catalytic sequence responsible for
mRNA cleavage. For this sequence, see U.S. Pat. No.
5,093,246, which is incorporated by reference herein in its
entirety. As such, within the scope of the invention are
15 engineered hammerhead motif ribozyme molecules that
specifically and efficiently catalyze endonucleolytic
cleavage of RNA sequences encoding target gene proteins.
Ribozyme molecules designed to catalytically cleave target or
pathway gene mRNA transcripts can also be used to prevent
20 translation of target or pathway gene mRNA and expression of
target or pathway gene. (See, e.g., PCT International
Publication WO90/11364, published October 4, 1990; Sarver et
al., 1990, Science 247:1222-1225). While ribozymes that
cleave mRNA at site specific recognition sequences can be
25 used to destroy target or pathway gene mRNAs, the use of
hammerhead ribozymes is preferred.

Hammerhead ribozymes cleave mRNAs at locations dictated
by flanking regions that form complementary base pairs with
the target mRNA. The sole requirement is that the target
30 mRNA have the following sequence of two bases: 5'-UG-3'.
The construction and production of hammerhead ribozymes is
well known in the art and is described more fully in Haseloff
and Gerlach, 1988, Nature, 334:585-591. Preferably the
ribozyme is engineered so that the cleavage recognition site
35 is located near the 5' end of the target or pathway gene
mRNA; i.e., to increase efficiency and minimize the

intracellular accumulation of non-functional mRNA
transcripts.

    The ribozymes of the present invention also include RNA
endoribonucleases (hereinafter "Cech-type ribozymes") such as
5 the one which occurs naturally in Tetrahymena Thermophila
(known as the IVS, or L-19 IVS RNA) and which has been
extensively described by Thomas Cech and collaborators (Zaug,
et al., 1984, Science, 224:574-578; Zaug and Cech, 1986,
Science, 231:470-475; Zaug, et al., 1986, Nature, 324:429-
10 433; published International patent application No. WO
88/04300 by University Patents Inc.; Been and Cech, 1986,
Cell, 47:207-216). The Cech-type ribozymes have an eight
base pair active site which hybridizes to a target RNA
sequence whereafter cleavage of the target RNA takes place.
15 The invention encompasses those Cech-type ribozymes which
target eight base-pair active site sequences that are present
in target or pathway gene.

    As in the antisense approach, the ribozymes can be
composed of modified oligonucleotides (e.g. for improved
20 stability, targeting, etc.) and should be delivered to cells
which express the target or pathway gene in vivo. A
preferred method of delivery involves using a DNA construct
"encoding" the ribozyme under the control of a strong
constitutive pol III or pol II promoter, so that transfected
25 cells will produce sufficient quantities of the ribozyme to
destroy endogenous target or pathway gene messages and
inhibit translation. Because ribozymes unlike antisense
molecules, are catalytic, a lower intracellular concentration
is required for efficiency.

30   In instances wherein the antisense, ribozyme, and/or
triple helix molecules described herein are utilized to
inhibit mutant gene expression, it is possible that the
technique can also efficiently reduce or inhibit the
transcription (triple helix) and/or translation (antisense,
35 ribozyme) of mRNA produced by normal target gene alleles that
the possibility can arise wherein the concentration of normal
target gene product present can be lower than is necessary

for a normal phenotype. In such cases, to ensure that
substantially normal levels of target gene activity are
maintained, therefore, nucleic acid molecules that encode and
express target gene polypeptides exhibiting normal target
5 gene activity can be introduced into cells via gene therapy
methods such as those described, below, in Section 5.9.2 that
do not contain sequences susceptible to whatever antisense,
ribozyme, or triple helix treatments are being utilized.
Alternatively, in instances whereby the target gene encodes
10 an extracellular protein, it can be preferable to
coadminister normal target gene protein in order to maintain
the requisite level of target gene activity.

Anti-sense RNA and DNA, ribozyme, and triple helix
molecules of the invention can be prepared by any method
15 known in the art for the synthesis of DNA and RNA molecules.
These include techniques for chemically synthesizing
oligodeoxyribonucleotides and oligoribonucleotides well known
in the art such as for example solid phase phosphoramidite
chemical synthesis. Alternatively, RNA molecules can be
20 generated by _in vitro_ and _in vivo_ transcription of DNA
sequences encoding the antisense RNA molecule. Such DNA
sequences can be incorporated into a wide variety of vectors
which incorporate suitable RNA polymerase promoters such as
the T7 or SP6 polymerase promoters. Alternatively, antisense
25 cDNA constructs that synthesize antisense RNA constitutively
or inducibly, depending on the promoter used, can be
introduced stably into cell lines.

Various well-known modifications to the DNA molecules
can be introduced as a means of increasing intracellular
30 stability and half-life. Possible modifications include, but
are not limited to, the addition of flanking sequences of
ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the
molecule or the use of phosphorothioate or 2' O-methyl rather
than phosphodiesterase linkages within the
35 oligodeoxyribonucleotide backbone.

Endogenous target and/or pathway gene expression can
also be reduced by inactivating or "knocking out" the target

and/or pathway gene or its promoter using targeted homologous recombination. (E.g., see Smithies et al., 1985, Nature 317:230-234; Thomas & Capecchi, 1987, Cell 51:503-512; Thompson et al., 1989 Cell 5:313-321; each of which is

5 incorporated by reference herein in its entirety). For example, a mutant, non-functional target and/or pathway gene (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous target and/or pathway gene (either the coding regions or regulatory regions of the

10 target and/or pathway gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express target and/or pathway gene in vivo. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the

15 target and/or pathway gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive target and/or pathway gene (e.g., see Thomas & Capecchi 1987 and Thompson 1989, supra). Such

20 techniques can also be utilized to generate T cell subpopulation-related disorder animal models. It should be noted that this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site in vivo using

25 appropriate viral vectors, e.g., herpes virus vectors.

Alternatively, endogenous target and/or pathway gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the target and/or pathway gene (i.e., the target and/or pathway

30 gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the target or pathway gene in target cells in the body. (See generally, Helene, C. 1991, Anticancer Drug Des., 6(6):569-84; Helene, C., et al., 1992, Ann, N.Y. Accad. Sci., 660:27-36; and

35 Maher, L.J., 1992, Bioassays 14(12):807-15). In yet another embodiment of the invention, the activity of target and/or pathway gene can be reduced using a "dominant negative"

approach. To this end, constructs which encode defective
target and/or pathway gene products can be used in gene
therapy approaches to diminish the activity of the target
and/or pathway gene product in appropriate target cells.

5

5.10.2. POSITIVE MODULATORY TECHNIQUES

As discussed above, successful treatment of certain
immune disorders can be brought about by techniques which
serve to increase the level of target gene expression or to
10 increase the activity of target gene product, or which, or
alternatively, serve to effectively increase the overall
number of cells belonging to a specific TH cell
subpopulation. As also discussed above, techniques which
serve to increase the level of target gene expression or to
15 increase the activity of target gene product can serve to
enhance the repair or resolution of ischemia reperfusion
injury to an organ or tissue. Thus, such techniques can also
be used to successfully treat ischemic disorders or injuries.

For example, compounds such as those identified through
20 assays described, above, in Section 5.8, which exhibit
positive modulatory activity can be used in accordance with
the invention to ameliorate certain TH cell subpopulation-
related disorder symptoms, or to treat ischemic disorders and
injuries. As discussed in Section 5.8, above, such molecules
25 can include, but are not limited to proteins or protein
fragments of the target gene product, including fragments
corresponding to one or more functional domains of the target
gene product (e.g., an extracellular domain, a transmembrane
domain, or a cytosolic domain) or portions thereof. Such
30 molecules can also include peptides representing soluble
extracellular portions of target gene product transmembrane
proteins, phosphopeptides, small organic or inorganic
molecules, or antibodies (including, for example, polyclonal,
monoclonal, humanized, anti-idiotypic, chimeric or single
35 chain antibodies, and FAb, F(ab')$_2$ and FAb expression library
fragments, and epitope-binding fragments thereof).

For example, a compound, such as a target gene protein,
can, at a level sufficient to ameliorate immune disorder
symptoms, be administered to a patient exhibiting such
symptoms. Such compounds can also be administered to a
5 patient having an ischemic disorder or injury at a level
sufficient to ameliorate the symptoms of the ischemic
disorder or injury. Any of the techniques discussed, below,
in Section 5.11, can be utilized for such administration.
One of skill in the art will readily know how to determine
10 the concentration of effective, non-toxic doses of the
compound, utilizing techniques such as those described,
below, in Section 5.11.1.

In another embodiment, fragments or peptides
representing a functional domain of a target gene product are
15 administered to an individual at sufficient dosages and such
that the fragments or peptides may enhance the target gene
product's activity in the individual, e.g., by mimicking the
function of the target gene product in vivo. For example, in
one preferred embodiment, fragments or peptides representing
20 a functional domain of the 200 gene product are administered
to a patient which enhance and/or mimic the activity of the
endogenous 200 gene product in that patient. Such 200 gene
fragments may therefore be used, e.g., to stimulate or
increase the repair of injury to tissue or organs resulting
25 from an ischemic injury or disorder.

The proteins and peptides which may be used in such
methods include synthetic (e.g., recombinant or chemically
synthesized) proteins and peptides, as well as naturally
occurring proteins and peptides. The proteins and peptides
30 may have both naturally occurring and/or non-naturally
occurring amino acid residues (e.g., D-amino acid residues)
and/or one or more non-peptide bonds (e.g., imino, ester,
hydrazide, semicarbazide, and azo bonds). The proteins or
peptides may also contain additional chemical groups (e.g.,
35 functional groups) present at the amino and/or carboxy
termini, such that, for example, the stability,
bioavailability, and/or inhibitory activity of the peptide is

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enhanced. Exemplary functional groups include hydrophobic groups (e.g., carbobenzoxyl, dansyl, and t-butyloxycarbonyl groups) an acetyl group, a 9-fluorenylmethoxy-carbonyl group, and macromolecular carrier groups (e.g., lipid-fatty acid

5 conjugates, polyethylene glycol, or carbohydrates) including peptide groups. Suitable dosages and formulations for administration of such peptides and proteins are also well known to those of skill in the art, and are described in Section 5.11 above.

10   In instances wherein the compound to be administered is a peptide compound, DNA sequences encoding the peptide compound can be directly administered to a patient exhibiting immune disorder symptoms, at a concentration sufficient to produce a level of peptide compound sufficient to ameliorate

15 the disorder symptoms. Any of the techniques discussed, below, in Section 5.11, which achieve intracellular administration of compounds, such as, for example, liposome administration, can be utilized for the administration of such DNA molecules. The DNA molecules can be produced, for

20 example, by well known recombinant techniques.
    In the case of peptides compounds which act extracellularly, the DNA molecules encoding such peptides can be taken up and expressed by any cell type, so long as a sufficient circulating concentration of peptide results for

25 the elicitation of a reduction in the immune disorder symptoms, or in a reduction of the symptoms of ischemic disorders and injuries. In the case of compounds which act intracellularly, the DNA molecules encoding such peptides must be taken up and expressed by the TH cell subpopulation

30 of interest at a sufficient level to bring about the reduction of immune disorders.
    Any technique which serves to selectively administer DNA molecules to the TH cell subpopulation of interest is, therefore, preferred, for the DNA molecules encoding

35 intracellularly acting peptides. In the case of asthma, for example, techniques for the selective administration of the

molecules to TH cell subpopulations residing within lung
tissue are preferred.

In cases wherein such molecules are administered to
treat an ischemia related disorder or injury, preferred
5 techniques are those which serve to selectively administer
DNA molecules to the affected organ or tissue. For example,
in the case of ischemic renal disease, techniques for the
selective administration of the molecules to cells within the
kidney are preferred.

10   In instances wherein the TH cell subpopulation-related
disorder involves an aberrant gene, patients can be treated
by gene replacement therapy. One or more copies of a normal
target gene or a portion of the gene that directs the
production of a normal target gene protein with target gene
15 function, can be inserted into cells, using vectors which
include, but are not limited to adenovirus, adeno-associated
virus, and retrovirus vectors, in addition to other particles
that introduce DNA into cells, such as liposomes.

Such gene replacement techniques can be accomplished
20 either in vivo or in vitro. As above, for genes encoding
extracellular molecules, the cell type expressing the target
gene is less important than achieving a sufficient
circulating concentration of the extracellular molecule for
the amelioration of immune disorders, or to ameliorate the
25 symptoms of ischemic disorders or injuries. Further, as
above, when the gene encodes a cell which acts
intracellularly or as a transmembrane molecule, the gene must
be expressed with the TH cell subpopulation cell type of
interest. Techniques which select for expression within the
30 cell type of interest are, therefore, preferred for this
latter class of target genes. In vivo, such techniques can,
for example, include appropriate local administration of
target gene sequences.

Additional methods which may be utilized to increase the
35 overall level of target and/or pathway gene expression and/or
target and/or pathway gene activity include the introduction
of appropriate target and/or pathway gene-expressing cells,

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preferably autologous cells, into a patient at positions and
in numbers which are sufficient to ameliorate the symptoms of
T cell subpopulation related disorders, or, alternatively, to
ameliorate the symptoms of ischemic disorders or injuries.

5 Such cells may be either recombinant or non-recombinant.
Among the cells which can be administered to increase the
overall level of target and/or pathway gene expression in a
patient are normal cells, which express the target and/or
pathway gene. The cells can be administered at the
10 anatomical site of expression, or as part of a tissue graft
located at a different site in the body. Such cell-based
gene therapy techniques are well known to those skilled in
the art, see, e.g., Anderson, et al., U.S. Patent No.
5,399,349; Mulligan & Wilson, U.S. Patent No. 5,460,959.

15   In vitro, target gene sequences can be introduced into
autologous cells. These cells expressing the target gene
sequence of interest can then be reintroduced, preferably by
intravenous administration, into the patient such that there
results an amelioration of the symptoms of the disorder.

20   Alternatively, for the amelioration of a TH cell
subpopulation-related disorder, TH cells belonging to a
specific TH cell subpopulation can be administered to a
patient such that the overall number of cells belonging to
that TH cell subpopulation relative to other TH cell
25 subpopulation cells is increased. Techniques for such TH
cell subpopulation augmentation are described, below, in
Section 5.10.3.2.

     5.10.3. NEGATIVE OR POSITIVE MODULATORY TECHNIQUES
30   Described herein are modulatory techniques which,
depending on the specific application for which they are
utilized, can yield either positive or negative responses
leading to the amelioration of immune disorders, including TH
cell subpopulation-related disorders. Further, the
35 modulatory techniques described in Section 5.10.3.1, below,
can also be used to treat ischemic disorders and injuries,
or, alternatively, to block or inhibit repair of ischemic

injuries depending on whether such modulation is positive or
negative. Thus, in appropriate instances, the procedures of
this Section can be used in conjunction with the negative
modulatory techniques described, above, in Section 5.9.1 or,
5 alternatively, in conjunction with the positive modulatory
techniques described, above, in Section 5.9.2.

5.10.3.1. ANTIBODY TECHNIQUES

Antibodies exhibiting modulatory capability can be
10 utilized to ameliorate immune disorders such as TH cell
subpopulation-related disorders, or to treat ischemic
disorders and injuries. Depending on the specific antibody,
the modulatory effect can be negative and can, therefore, by
utilized as part of the techniques described, above, in
15 Section 5.9.1, or can be positive, and can, therefore, be
used in conjunction with the techniques described, above, in
Section 5.9.2.

An antibody having negative modulatory capability refers
to an antibody which specifically binds to and interferes
20 with the action of a protein. In the case of an
extracellular receptor, for example, such an antibody would
specifically bind the extracellular domain of the receptor in
a manner which does not activate the receptor but which
disrupts the ability of the receptor to bind its natural
25 ligand. For example, antibodies directed against the
extracellular domains of genes 103 or 200 can function as
such negative modulators. Additionally, antibodies directed
against one or more of the 10 gene product extracellular
domains can function in a negative modulatory manner. Such
30 antibodies can be generated using standard techniques
described in Section 5.6, above, against full length wild
type or mutant proteins, or against peptides corresponding to
portions of the proteins. The antibodies include but are not
limited to polyclonal, monoclonal, FAb fragments, single
35 chain antibodies, chimeric antibodies, and the like.

An antibody having positive modulatory capability refers
to an antibody which specifically binds to a protein and, by

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binding, serves to, either directly or indirectly, activate
the function of the protein which it recognizes. For
example, an antibody can bind to the extracellular portion of
a transmembrane protein in a manner which causes the

5 transmembrane protein to function as though its endogenous
ligand was binding, thus activating, for example, a signal
transduction' pathway. antibodies can be generated using
standard techniques described in Section 5.6, above, against
full length wild type or mutant proteins, or against peptides

10 corresponding to portions of the proteins. The antibodies
include but are not limited to polyclonal, monoclonal, FAb
fragments, single chain antibodies, chimeric antibodies, and
the like.

   In instances where the protein, such as a target gene

15 protein, to which the antibody is directed is intracellular
and whole antibodies are used, internalizing antibodies can
be preferred. However, lipofectin or liposomes can be used
to deliver the antibody or a fragment of the Fab region which
binds to the gene product epitope into cells. Where

20 fragments of the antibody are used, the smallest inhibitory
fragment which binds to the protein's binding domain is
preferred. For example, peptides having an amino acid
sequence corresponding to the domain of the variable region
of the antibody that binds to the protein can be used. Such

25 peptides can be synthesized chemically or produced via
recombinant DNA technology using methods well known in the
art (e.g., see Creighton, 1983, supra; and Sambrook et al.,
1989, above). Alternatively, single chain antibodies, such
as neutralizing antibodies, which bind to intracellular

30 epitopes can also be administered. Such single chain
antibodies can be administered, for example, by expressing
nucleotide sequences encoding single-chain antibodies within
the target cell population by utilizing, for example,
techniques such as those described in Marasco et al.

35 (Marasco, W. et al., 1993, Proc. Natl. Acad. Sci. USA
90:7889-7893).

In instances where the protein to which the antibody is directed is extracellular, or is a transmembrane protein, any of the administration techniques described, below in Section 5.11 which are appropriate for peptide administration can be utilized to effectively administer the antibodies to their site of action.

5.10.3.2. METHODS FOR INCREASING OR DECREASING SPECIFIC TH CELL SUBPOPULATION CONCENTRATIONS

Techniques described herein can be utilized to either deplete or augment the total number of cells belonging to a given TH cell subpopulation, thus effectively increasing or decreasing the ratio of the TH cell subpopulation of interest to other TH cell subpopulations. Specifically, separation techniques are described which can be used to either deplete or augment the total number of cells present within a TH cell subpopulation, and, further, targeting techniques are described which can be utilized to deplete specific TH cell subpopulations.

Depending on the particular application, changing the number of cells belonging to a TH cell subpopulation can yield either stimulatory or inhibitory responses leading to the amelioration of TH cell subpopulation disorders. Thus, in appropriate instances, the procedures of this Section can be used in conjunction with the inhibitory techniques described, above, in Section 5.9.1. or, alternatively, in conjunction with the stimulatory techniques described, above, in Section 5.9.2.

The separation techniques described herein are based on the presence or absence of specific cell surface markers, preferably transmembrane markers. Such markers can include, but are not limited to, the TH2-specific 103 gene product extracellular domain markers, the TH1-specific 200 gene product extracellular domain markers and the TH inducible 10 gene product extracellular domain markers.

In instances wherein the goal of the separation is to increase or augment the number of cells belonging to a

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specific TH cell subpopulation, the antibodies used can also
be specific to surface markers present on undifferentiated or
partially undifferentiated TH cells. After separation, and
purification of such undifferentiated or partially

5 differentiated TH cells, the cells can be cultured in
physiological buffer or culture medium and induced to
differentiate by culturing in the presence of appropriate
factors. For example, IL-4 can be added to induce the TH
cells to differentiate into TH2 cells, while the cytokine IL-

10 12 can be added to induce the TH cells to differentiate into
TH1 cells. After differentiation, cells can be washed,
resuspended in, for example, buffered saline, and
reintroduced into a patient via, preferably, intravenous
administration.

15   Separation techniques can be utilized which separate and
purify cells, in vitro, from a population of cells, such as
hematopoietic cells autologous to the patient being treated.
An initial TH cell subpopulation-containing population of
cells, such as hematopoietic cells, can be obtained using

20 standard procedures well known to those of skill in the art.
Peripheral blood can be utilized as one potential starting
source for such techniques, and can, for example, be obtained
via venipuncture and collection into heparinized tubes.

Once the starting source of autologous cells is

25 obtained, the T cells, such as TH1 or TH2 cells, can be
removed, and thus selectively separated and purified, by
various methods which utilize antibodies which bind specific
markers present on the T cell population of interest, while
absent on other cells within the starting source. These

30 techniques can include, for example, flow cytometry using a
fluorescence activated cell sorter (FACS) and specific
fluorochromes, biotin-avidin or biotin-streptavidin
separations using biotin conjugated to cell surface marker-
specific antibodies and avidin or streptavidin bound to a

35 solid support such as affinity column matrix or plastic
surfaces or magnetic separations using antibody-coated
magnetic beads.

Separation via antibodies for specific markers can be by
negative or positive selection procedures. In negative
separation, antibodies are used which are specific for
markers present on undesired cells. For example, in the case
5 of a TH1 cell subpopulation-related disorder wherein it would
be desirable to deplete the number of TH1 cells, such
antibodies could be directed to the extracellular domain of
the 200 gene product. Alternatively, in the case of TH2 cell
subpopulation-related disorders wherein it would be desirable
10 to deplete the number of TH1 cells, such antibodies could be
directed to the extracellular domain of the 103 gene product.
Cells bound by an antibody to such a cell surface marker can
be removed or lysed and the remaining desired mixture
retained.

15   In positive separation, antibodies specific for markers
present on the desired cells of interest. For example, in
the case of a TH1 cell subpopulation-related disorder wherein
it would be desirable to increase the number of TH1 cells,
such antibodies could be directed to the extracellular domain
20 of the 200 gene product. Alternatively, in the case of TH2
cell subpopulation-related disorders wherein it would be
desirable to increase the number of TH1 cells, such
antibodies could be directed to the extracellular domain of
the 103 gene product. Cells bound by the antibody are
25 separated and retained. It will be understood that positive
and negative separations can be used substantially
simultaneously or in a sequential manner.

A common technique for antibody based separation is the
use of flow cytometry such as by a florescence activated cell
30 sorter (FACS). Typically, separation by flow cytometry is
performed as follows. The suspended mixture of cells are
centrifuged and resuspended in media. Antibodies which are
conjugated to fluorochrome are added to allow the binding of
the antibodies to specific cell surface markers. The cell
35 mixture is then washed by one or more centrifugation and
resuspension steps. The mixture is run through a FACS which
separates the cells based on different fluorescence

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characteristics. FACS systems are available in varying
levels of performance and ability, including multi-color
analysis. The facilitating cell can be identified by a
characteristic profile of forward and side scatter which is
5 influenced by size and granularity, as well as by positive
and/or negative expression of certain cell surface markers.

Other separation techniques besides flow cytometry can
also provide fast separations. One such method is biotin-
avidin based separation by affinity chromatography.
10 Typically, such a technique is performed by incubating cells
with biotin-coupled antibodies to specific markers, such as,
for example, the transmembrane protein encoded by the 103
gene described herein, followed by passage through an avidin
column. Biotin-antibody-cell complexes bind to the column
15 via the biotin-avidin interaction, while other cells pass
through the column. The specificity of the biotin-avidin
system is well suited for rapid positive separation.
Multiple passages can ensure separation of a sufficient level
of the TH cell subpopulation of interest.
20   In instances whereby the goal of the separation
technique is to deplete the overall number of cells belonging
to a TH cell subpopulation, the cells derived from the
starting source of cells which has now been effectively
depleted of TH cell subpopulation cells can be reintroduced
25 into the patient. Such a depletion of the TH cell
subpopulation results in the amelioration of TH cell
subpopulation-related disorders associated with the activity
or overactivity of the TH cell subpopulation. Reintroduction
of the TH cell subpopulation-depleted cells can be
30 accomplished by washing the cells, resuspending in, for
example, buffered saline, and intravenously administering the
cells into the patient.

If cell viability and recovery are sufficient, TH cell
subpopulation-depleted cells can be reintroduced into
35 patients immediately subsequent to separation.
Alternatively, TH cell subpopulation-depleted cells can be
cultured and expanded ex vivo prior to administration to a

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patient. Expansion can be accomplished via well known
techniques utilizing physiological buffers or culture media
in the presence of appropriate expansion factors such as
interleukins and other well known growth factors.

5   In instances whereby the goal of the separation
technique is to augment or increase the overall number of
cells belonging to a TH cell subpopulation, cells derived
from the purified TH cell subpopulation cells can be
reintroduced into the patient, thus resulting in the
10 amelioration of TH cell subpopulation-related disorders
associated with an under activity of the TH cell
subpopulation.

        The cells to be reintroduced will be cultured and
expanded ex vivo prior to reintroduction. Purified TH cell
15 subpopulation cells can be washed, suspended in, for example,
buffered saline, and reintroduced into the patient via
intravenous administration.

        Cells to be expanded can be cultured, using standard
procedures, in the presence of an appropriate expansion agent
20 which induces proliferation of the purified TH cell
subpopulation. Such an expansion agent can, for example, be
any appropriate cytokine, antigen, or antibody. In the case
of TH2 cells, for example, the expansion agent can be IL-4,
while for TH1 cells, the expansion agent can, for example, be
25 IL-12.

        Prior to being reintroduced into a patient, the purified
cells can be modified by, for example, transformation with
gene sequences encoding gene products of interest. Such gene
products should represent products which enhance the activity
30 of the purified TH cell subpopulation or, alternatively,
represent products which repress the activity of one or more
of the other TH cell subpopulations. Cell transformation and
gene expression procedures are well known to those of skill
in the art, and can be as those described, above, in Section
35 5.5.

        Well known targeting methods can, additionally, be
utilized in instances wherein the goal is to deplete the

number of cells belonging to a specific TH cell
subpopulation. Such targeting methods can be in vivo or in
vitro, and can involve the introduction of targeting agents
into a population of cells such that the targeting agents
5 selectively destroy a specific subset of the cells within the
population. In vivo administration techniques which can be
followed for such targeting agents are described, below, in
Section 5.11.

Targeting agents generally comprise, first, a targeting
10 moiety which, in the current instance, causes the targeting
agent to selectively associate with a specific TH cell
subpopulation. The targeting agents generally comprise,
second, a moiety capable of destroying a cell with which the
targeting agent has become associated.

15   Targeting moieties can include, but are not limited to,
antibodies directed to cell surface markers found
specifically on the TH cell subpopulation being targeted, or,
alternatively, to ligands, such as growth factors, which bind
receptor-type molecules found exclusively on the targeted TH
20 cell subpopulation.

In the case of TH2 cells, for example, such a targeting
moiety can represent an antibody directed against the
extracellular portion of the 103 gene product described
herein, or can, alternatively, represent a ligand specific
25 for this receptor-type TH2 specific molecule. In the case of
TH1 cells, for example, such a targeting moiety can represent
an antibody directed against the extracellular portion of the
200 gene product described herein, or can, alternatively,
represent a ligand specific for this receptor-type TH1
30 specific molecule.

Destructive moieties include any moiety capable of
inactivating or destroying a cell to which the targeting
agent has become bound. For example, a destructive moiety
can include, but it is not limited to cytotoxins or
35 radioactive agents. Cytotoxins include, for example, plant-,
fungus-, or bacteria-derived toxins, with deglycosylated

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Ricin A chain toxins being generally preferred due to their
potency and lengthy half-lives.

5.11. PHARMACEUTICAL PREPARATIONS AND METHODS OF ADMINISTRATION

The compounds, nucleic acid sequences and TH cell
subpopulation cell described herein can be administered to a
patient at therapeutically effective doses to treat or
ameliorate immune disorders, e.g., TH cell subpopulation-
related disorders. A therapeutically effective dose refers
to that amount of a compound or TH cell subpopulation
sufficient to result in amelioration of the immune disorder
symptoms of the immune disorder symptoms, or alternatively,
to that amount of a nucleic acid sequence sufficient to
express a concentration of gene product which results in the
amelioration of the TH cell subpopulation-related disorders
or of other immune disorders.

5.11.1. EFFECTIVE DOSE

Toxicity and therapeutic efficacy of compounds can be
determined by standard pharmaceutical procedures in cell
cultures or experimental animals, e.g., for determining the
LD_{50} (the dose lethal to 50% of the population) and the ED_{50}
(the dose therapeutically effective in 50% of the
population). The dose ratio between toxic and therapeutic
effects is the therapeutic index and it can be expressed as
the ratio LD_{50}/ED_{50}. Compounds which exhibit large therapeutic
indices are preferred. While compounds that exhibit toxic
side effects can be used, care should be taken to design a
delivery system that targets such compounds to the site of
affected tissue in order to minimize potential damage to
uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and
animal studies can be used in formulating a range of dosage
for use in humans. The dosage of such compounds lies
preferably within a range of circulating concentrations that
include the ED_{50} with little or no toxicity. The dosage can

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vary within this range depending upon the dosage form
employed and the route of administration utilized. For any
compound used in the method of the invention, the
therapeutically effective dose can be estimated initially
5 from cell culture assays. A dose can be formulated in animal
models to achieve a circulating plasma concentration range
that includes the IC_{50} (*i.e.*, the concentration of the test
compound which achieves a half-maximal inhibition of
symptoms) as determined in cell culture. Such information
10 can be used to more accurately determine useful doses in
humans. Levels in plasma can be measured, for example, by
high performance liquid chromatography.

As defined herein, a therapeutically effective amount of
antibody, protein, or polypeptide (*i.e.*, an effective dose or
15 effective dosage) ranges from about 0.001 to 30 mg/kg of body
weight, preferably about 0.01 to 25 mg/kg body weight, more
preferably about 0.1 to 20 mg/kg body weight, and even more
preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4
to 7 mg/kg, or 5 to 6 mg/kg body weight.
20   The skilled artisan will appreciate that certain factors
may influence the dosage required to effectively treat a
subject, including but not limited to the severity of the
disease or disorder, previous treatments, the general health
and/or age of the subject, and other diseases present.
25 Moreover, treatment of a subject with a therapeutically
effective amount of a protein, polypeptide or antibody can
include a single treatment or, preferably, can include a
series of treatments. In a preferred example, a subject is
treated with antibody, protein, or polypeptide in the range
30 of between about 0.1 to 20 mg/kg body weight one time per
week for between about 1 to 10 weeks, preferably between 2 to
8 weeks, more preferably between about 3 to 7 weeks, and even
more preferably for about 4, 5, or 6 weeks. It will also be
appreciated that the effective dosage of antibody, protein or
35 polypeptide used for treatment may increase or decrease over
the course of a particular treatment. Changes in dosage may

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also be apparent to one skilled in the art from the results
of diagnostic assays as described herein.

5.11.2. FORMULATIONS AND USE

5   Pharmaceutical compositions for use in accordance with
the present invention can be formulated in conventional
manner using one or more physiologically acceptable carriers
or excipients.

Thus, the compounds and their physiologically acceptable
10 salts and solvents can be formulated for administration by
inhalation or insufflation (either through the mouth or the
nose) or oral, buccal, parenteral, subuctaneous,
intraperitoneal, intrapulmonary, intranasal, intralesional,
vaginal or rectal administration.

15   For oral administration, the pharmaceutical compositions
can take the form of, for example, tablets or capsules
prepared by conventional means with pharmaceutically
acceptable excipients such as binding agents (e.g.,
pregelatinised maize starch, polyvinylpyrrolidone or
20 hydroxypropyl methylcellulose); fillers (e.g., lactose,
microcrystalline cellulose or calcium hydrogen phosphate);
lubricants (e.g., magnesium stearate, talc or silica);
disintegrants (e.g., potato starch or sodium starch
glycolate); or wetting agents (e.g., sodium lauryl sulphate).
25 The tablets can be coated by methods well known in the art.
Liquid preparations for oral administration can take the form
of, for example, solutions, syrups or suspensions, or they
can be presented as a dry product for constitution with water
or other suitable vehicle before use. Such liquid
30 preparations can be prepared by conventional means with
pharmaceutically acceptable additives such as suspending
agents (e.g., sorbitol syrup, cellulose derivatives or
hydrogenated edible fats); emulsifying agents (e.g., lecithin
or acacia); non-aqueous vehicles (e.g., almond oil, oily
35 esters, ethyl alcohol or fractionated vegetable oils); and
preservatives (e.g., methyl or propyl-p-hydroxybenzoates or
sorbic acid). The preparations can also contain buffer

salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration can be suitably formulated to give controlled release of the active compound.

5   For buccal administration the compositions can take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered

10 in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit

15 can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

20   The compounds can be formulated for parenteral administration (i.e., intravenous or intramuscular) by injection, via, for example, bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, e.g., in ampoules or in multi-dose

25 containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form

30 for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. It is preferred that the TH cell subpopulation cells be introduced into patients via intravenous administration.

The compounds can also be formulated in rectal

35 compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds can also be formulated as a depot preparation. Such long acting formulations can be administered by implantation (for example subcutaneously or intramuscularly) 5 or by intramuscular injection. Thus, for example, the compounds can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble 10 salt.

Intralesional administration can comprise, for example, perfusing or contacting a graft or organ with a composition prior to transplantation.

The compositions can, if desired, be presented in a pack 15 or dispenser device which can contain one or more unit dosage forms containing the active ingredient. The pack can for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device can be accompanied by instructions for administration.

20

5.11.3. PHARMACEUTICAL PREPARATION AND ADMINISTRATION OF ANTIBODIES

Antibodies which specifically bind to target gene products of the invention and thereby modulate their activity 25 can also be administered to a patient at therapeutically effective doeses to treat or ameliorate immune disorders, or to treat or ameliorate ischemic disorders or injuries. For example, Section 13, below, demonstrates the use of anti-200 gene product antibodies to block recovery of kidney tissue 30 from ischemia reperfusion injury.

Antibodies of the invention are administered by any suitable means, including those described in Section 5.12.2, above. In addition, antibody to a target gene product of the invention is suitably administered by pulse infusion, 35 particularly with declining doses of the antibody. Preferably, the dosing is administered by injections, most preferably by intravenous or subcutaneous injections,

depending in part on whether the administration is brief or chronic.

The appropriate dosage of antibody will depend on the type of disease to be treated, the severity and course of the
5 disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody may suitably administered to the patient at one time or, more
10 preferably, over a series of treatments.

As a general proposition, the initial pharmaceutically effective amount of antibody administered parenterally will be in the range of about 0.1 to 20 mg/kg of patient body weight per day, with the typical initial range being 0.3 to
15 15 mg/kg of patient body weight per day. Where the subsequent dosing is less than 100% of initial dosing, such subsequent dosing is calculated on the basis of daily dosing. Thus, for example, if the dosing regimen consists of daily injections of 2 mg/kg of patient body weight per day for two
20 weeks followed by a biweekly dose of 0.5 mg/kg of patient body weight per day for 99 days, this would amount to a subsequent dose of about 1.8% of the initial dose calculated on a daily basis (*i.e.*, 2/day/100% = 0.5/14 days/x%, x=1.8%). Preferably, the subsequent dosing is less than about 50%,
25 more preferably, less than about 25%, still more preferably, less than about 10%, and still more preferably, less than about 5%. Most preferably, the subsequent dosing is less than about 2% of the initial dosing.

Overall, dosage ranges to be administered will,
30 preferably, range from about 1 µg/kg to about 100 mg/kg, 1 µg/kg to about 15 mg/kg, or about 1 µg/kg, to about 2.0 mg/kg body weight.

The preferred scheduling is that the initial dosing is administered no less frequently than daily, and up to an
35 including continuously by infusion. More preferably, depending on the specific disorder or injury, the initial daily dosing is administered for at least about one week, and

preferably at least about two weeks. To obtain the most
efficacious results, the inital dosing is preferably given as
close to the first sign, diagnosis, appearance, or occurrence
of the disorder as possible, or, particular in the case of
5 immune disorders, during remission of the disorder.

Subsequent dosing is preferably administered
periodically no more about once a week. More preferably, the
subsequent dosing is administered no more than once biweekly.
Subsequent dosing is typically administered for at least
10 about five weeks, and preferably for at least about 10 weeks,
after the initial dosing is terminated.

Exemplary dosing regimens are disclosed in Jardieu,
P.M., and Presta, L.G., 1998, WO 98/23761; and in Jardieu,
P.M., and Presta, L.G., 1994, WO 94/04188, each of which is
15 incorporated herein, by reference, in its entirety.

5.12.   DIAGNOSTIC AND MONITORING TECHNIQUES

A variety of methods can be employed for the diagnosis
of immune disorders, e.g., TH cell subpopulation-related
20 disorders, predisposition to such immune disorders, for
monitoring the efficacy of anti-immune disorder compounds
during, for example, clinical trials and for monitoring
patients undergoing clinical evaluation for the treatment of
such disorders. Further, a number of methods can be utilized
25 for the detection of activated immune cells, e.g., activated
members of TH cell subpopulations.

Such methods can, for example, utilize reagents such as
the fingerprint gene nucleotide sequences described in
Sections 5.1, and antibodies directed against differentially
30 expressed and pathway gene peptides, as described, above, in
Sections 5.5 (peptides) and 5.6 (antibodies). Specifically,
such reagents can be used, for example, for: 1) the detection
of the presence of target gene expression, target gene
mutations, the detection of either over- or under-expression
35 of target gene mRNA relative to the non-immune disorder state
or relative to an unactivated TH cell subpopulation; 2) the
detection of either an over- or an underabundance of target

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gene product relative to the non-immune disorder state or
relative to the unactivated TH cell subpopulation state; and
3) the identification of specific TH cell subpopulation cells
(e.g., TH cells involved in an immune disorder, or activated
5 TH cells) within a mixed population of cells.

The methods described herein can be performed, for
example, by utilizing pre-packaged diagnostic kits comprising
at least one specific fingerprint gene nucleic acid or anti-
fingerprint gene antibody reagent described herein, which can
10 be conveniently used, e.g., in clinical settings, to diagnose
patients exhibiting TH1- or TH2-related abnormalities.

Any cell type or tissue, preferably TH cells, in which
the fingerprint gene is expressed can be utilized in the
diagnostics described below.

15   Among the methods which can be utilized herein are
methods for monitoring the efficacy of compounds in clinical
trials for the treatment of immune disorders. Such compounds
can, for example, be compounds such as those described,
above, in Section 5.9. Such a method comprises detecting, in
20 a patient sample, a gene transcript or gene product which is
differentially expressed in a TH cell subpopulation in an
immune disorder state relative to its expression in the TH
cell subpopulation when the cell subpopulation is in a
normal, or non-immune disorder, state.

25   Any of the nucleic acid detection techniques described,
below, in Section 5.12.1 or any of the peptide detection
techniques described, below, in Section 5.12.2 can be used to
detect the gene transcript or gene product which is
differentially expressed in the immune disorder TH cell
30 subpopulation relative to its expression in the normal, or
non-immune disorder, state.

During clinical trials, for example, the expression of a
single fingerprint gene, or alternatively, the fingerprint
pattern of a TH cell subpopulation, can be determined for the
35 TH cell subpopulation in the presence or absence of the
compound being tested. The efficacy of the compound can be
followed by comparing the expression data obtained to the

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corresponding known expression patterns for the TH cell subpopulation in a normal, non-immune disorder state. Compounds exhibiting efficacy are those which alter the single fingerprint gene expression and/or the fingerprint

5 pattern of the immune disorder TH cell subpopulation to more closely resemble that of the normal, non-immune disorder TH cell subpopulation.

The detection of the product or products of genes differentially expressed in a TH cell subpopulation in an

10 immune disorder state relative to their expression in the TH cell subpopulation when the cell subpopulation is in a normal, or non-immune disorder, state can also be used for monitoring the efficacy of potential anti-immune disorder compounds during clinical trials. During clinical trials,

15 for example, the level and/or activity of the products of one or more such differentially expressed genes can be determined for the TH cell subpopulation in the presence or absence of the compound being tested. The efficacy of the compound can be followed by comparing the protein level and/or activity

20 data obtained to the corresponding known levels/activities for the TH cell subpopulation in a normal, non-immune disorder state. Compounds exhibiting efficacy are those which alter the pattern of the immune disorder TH cell subpopulation to more closely resemble that of the normal,

25 non-immune disorder TH cell subpopulation.

Given the TH2-specific nature of the 103 gene, the detection of 103 gene transcripts and/or products can be particularly suitable for monitoring the efficacy of compounds in clinical trials for the treatment of TH2 cell

30 subpopulation-related immune disorders such as, for example, asthma or allergy.

The expression patterns of the 105, 106 and 200 genes in TH1 cell subpopulations relative to TH2 cell subpopulations can make the detection of transcripts and/or products of

35 these genes particularly suitable for monitoring the efficacy of compounds in clinical trials for the treatment of TH1 cell

subpopulation-related immune disorders such as, for example,
multiple sclerosis, psoriasis or insulin dependent diabetes.

Among the additional methods which can be utilized
herein are methods for detecting TH cell responsiveness, for

5 example, responsiveness to antigen, and for detecting
activated immune cells, e.g., activated members of TH cell
subpopulations. Detection methods such as these are
important in that many immune disorders involve inappropriate
rather than insufficient immune responses. Such detection

10 methods can be used, for example, to detect a predisposition
to an immune disorder.

Methods for detecting TH cell responsiveness and/or
activation can comprise, for example, detecting in a TH cell
sample a gene transcript or product which is differentially

15 expressed in TH cell subpopulation which is in an activated
or responsive state (e.g., a state in which the TH cell
subpopulation has been exposed to antigen), relative to a TH
cell subpopulation which is in an unactivated or
nonresponsive state.

20   Any of the nucleic acid detection techniques described,
below, in Section 5.12.1 or any of the peptide detection
techniques described, below, in Section 5.12.2 can be used to
detect such a differentially expressed gene transcript or
gene product.

25   In addition to diagnostic uses, such techniques can also
be utilized as part of methods for identifying compounds
which alter the cellular expression of one or more of the
differentially expressed genes described herein, or as part
of methods for identifying compounds which alter the cellular

30 and/or secreted level of product produced by the
differentially expressed genes described herein.

By way of example, and not by way of limitation, such
techniques can be used to identify compounds which alter the
level of expression of the 103 gene or the level of 103 gene

35 product present in a cell. Such methods can include, for
example, contacting a T cell with a compound, measuring the
level of 103 gene expression in the cell (or the level of 103

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gene product in the cell), then comparing the level obtained
to that of a cell not exposed to the compound. The T cells
used herein can include, for example, TH0, TH1 or TH2 cells.
            Such methods can furthe include stimulating the cells,
5 for example, stimulating the cells prior to contacting the
cells with the compound. Among the methods for stimulation
are stimulation via anti-CD3 antibody stimulation.
            Such methods can be performed such that the cell
contacted is presented within a non-human mammal, for
10 example, a mouse. Further, among the non-human mammals which
can be utilized as part of these methods are ones which
exhibit symptoms of a T cell-related disorder (such as, for
example a TH2-related disorder, e.g., asthma), and contacting
the cell with the compound can ameliorate symptoms of the
15 disorder.
            The TH2-specific nature of the 103 gene can make the
detection of its gene transcripts and/or products
particularly suitable for detecting activation and/or
responsiveness of TH2 cells. Further, the TH1-specific
20 nature of the 105, 106 and 200 genes can make the detection
of transcripts and/or products of these genes particularly
suitable for the detection of TH1 activation and/or
responsiveness.

25   5.12.1.   DETECTION OF FINGERPRINT GENE NUCLEIC ACIDS
            DNA or RNA from the cell type or tissue to be analyzed
can easily be isolated using procedures which are well known
to those in the art. Diagnostic procedures can also be
performed "in situ" directly upon, for example tissue
30 sections (fixed and/or frozen) of patient tissue obtained
from biopsies or resections, such that no nucleic acid
purification is necessary. Nucleic acid reagents such as
those described in Section 5.4 can be used as probes and/or
primers for such in situ procedures (see, for example, Nuovo,
35 G.J., 1992, "PCR In Situ Hybridization: Protocols and
Applications", Raven Press, NY). Expression of specific
cells within a population of cells can also be determined,

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via, for example, in situ techniques such as those described above, or by standard flow cytometric techniques.

Fingerprint gene nucleotide sequences, either RNA or DNA, can, for example, be used in hybridization or

5 amplification assays of biological samples to detect TH cell subpopulation-related disorder gene structures and expression. Such assays can include, but are not limited to, Southern or Northern analyses, single stranded conformational polymorphism analyses, in situ hybridization assays, and

10 polymerase chain reaction analyses. Such analyses can reveal both quantitative aspects of the expression pattern of the fingerprint gene, and qualitative aspects of the fingerprint gene expression and/or gene composition. That is, such techniques can detect not only the presence of gene

15 expression, but can also detect the amount of expression, particularly which specific cells are expressing the gene of interest, and can, further, for example, detect point mutations, insertions, deletions, chromosomal rearrangements, and/or activation or inactivation of gene expression.

20   Diagnostic methods for the detection of fingerprint gene-specific nucleic acid molecules can involve for example, contacting and incubating nucleic acids, derived from the cell type or tissue being analyzed, with one or more labeled nucleic acid reagents as are described in Section 5.4, under

25 conditions favorable for the specific annealing of these reagents to their complementary sequences within the nucleic acid molecule of interest. Preferably, the lengths of these nucleic acid reagents are at least 15 to 30 nucleotides. After incubation, all non-annealed nucleic acids are removed

30 from the nucleic acid:fingerprint molecule hybrid. The presence of nucleic acids from the cell type or tissue which have hybridized, if any such molecules exist, is then detected. Using such a detection scheme, the nucleic acid from the tissue or cell type of interest can be immobilized,

35 for example, to a solid support such as a membrane, or a plastic surface such as that on a microtiter plate or polystyrene beads. In this case, after incubation, non-

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annealed, labeled nucleic acid reagents of the type described
in Section 5.4 are easily removed. Detection of the
remaining, annealed, labeled fingerprint nucleic acid
reagents is accomplished using standard techniques well-known
5 to those in the art.

Alternative diagnostic methods for the detection of
fingerprint gene specific nucleic acid molecules can involve
their amplification, e.g., by PCR (the experimental
embodiment set forth in Mullis, K.B., 1987, U.S. Patent No.
10 4,683,202), ligase chain reaction (Barany, F., 1991, Proc.
Natl. Acad. Sci. USA 88:189-193), self sustained sequence
replication (Guatelli, J.C. et al., 1990, Proc. Natl. Acad.
Sci. USA 87:1874-1878), transcriptional amplification system
(Kwoh, D.Y et al., 1989, Proc. Natl. Acad. Sci. USA 86:1173-
15 1177), Q-Beta Replicase (Lizardi, P.M. et al., 1988,
Bio/Technology 6:1197), or any other nucleic acid
amplification method, followed by the detection of the
amplified molecules using techniques well known to those of
skill in the art. These detection schemes are especially
20 useful for the detection of nucleic acid molecules if such
molecules are present in very low numbers.

In one embodiment of such a detection scheme, a cDNA
molecule is obtained from an RNA molecule of interest (e.g.,
by reverse transcription of the RNA molecule into cDNA).
25 Cell types or tissues from which such RNA can be isolated
include any tissue in which wild type fingerprint gene is
known to be expressed, including, but not limited, to TH0,
TH1 and/or TH2 cell type-containing tissues. A sequence
within the cDNA is then used as the template for a nucleic
30 acid amplification reaction, such as a PCR amplification
reaction, or the like. The nucleic acid reagents used as
synthesis initiation reagents (e.g., primers) in the reverse
transcription and nucleic acid amplification steps of this
method are chosen from among the fingerprint gene nucleic
35 acid reagents described in Section 5.4. The preferred
lengths of such nucleic acid reagents are at least 9-30
nucleotides. For detection of the amplified product, the

nucleic acid amplification can be performed using
radioactively or non-radioactively labeled nucleotides.
Alternatively, enough amplified product can be made such that
the product can be visualized by standard ethidium bromide
5 staining or by utilizing any other suitable nucleic acid
staining method.

In addition to methods which focus primarily on the
detection of one fingerprint nucleic acid sequence,
fingerprint patterns can also be assessed in such detection
10 schemes. Fingerprint patterns, in this context, contain the
pattern of mRNA expression of a series (*i.e.*, at least two
and up to the total number present) of fingerprint genes
obtained for a given tissue or cell type under a given set of
conditions. Such conditions can include, for example, TH
15 cell subpopulation-related disorders, and conditions relevant
to processes involved in the differentiation, maintenance and
effector function of TH cell subpopulations.

TH1-related disorders can include, for example, chronic
inflammatory diseases and disorders, such as Crohn's disease,
20 reactive arthritis, including Lyme disease, insulin-dependent
diabetes, organ-specific autoimmunity, including multiple
sclerosis, Hashimoto's thyroiditis and Grave's disease,
contact dermatitis, psoriasis, graft rejection, graft versus
host disease and sarcoidosis. TH2-related disorders can
25 include, for example, atopic conditions, such as asthma and
allergy, including allergic rhinitis, gastrointestinal
allergies, including food allergies, eosinophilia,
conjunctivitis, glomerular nephritis, certain pathogen
susceptibilities such as helminthic (*e.g.*, leishmaniasis) and
30 certain viral infections, including HIV, and bacterial
infections, including tuberculosis and lepromatous leprosy.

Fingerprint patterns can be generated, for example, by
utilizing a differential display procedure, as discussed,
above, in Section 5.1.1.2, Northern analysis and/or RT-PCR.
35 Any of the gene sequences described, above, in Section 3.2.1
can be used as probes and/or RT-PCR primers for the
generation and corroboration of such fingerprint patterns.

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5.12.2. DETECTION OF TARGET GENE PEPTIDES

Antibodies directed against wild type or mutant
fingerprint gene peptides, which are discussed, above, in
Section 5.6, can also be used as TH cell subpopulation-
5 related disorder diagnostics and prognostics, as described,
for example, herein. Such diagnostic methods, can be used to
detect fingerprint gene product, abnormalities in the level
of fingerprint gene protein expression, or abnormalities in
the structure and/or temporal, tissue, cellular, or
10 subcellular location of fingerprint gene protein. Structural
differences can include, for example, differences in the
size, electronegativity, or antigenicity of the mutant
fingerprint gene protein relative to the normal fingerprint
gene protein.

15   Protein from the tissue or cell type to be analyzed can
easily be isolated using techniques which are well known to
those of skill in the art. The protein isolation methods
employed herein can, for example, be such as those described
in Harlow and Lane (Harlow, E. and Lane, D., 1988,
20 "Antibodies: A Laboratory Manual", Cold Spring Harbor
Laboratory Press, Cold Spring Harbor, New York), which is
incorporated herein by reference in its entirety.

        Preferred diagnostic methods for the detection of wild
type or mutant fingerprint gene peptide molecules can
25 involve, for example, immunoassays wherein fingerprint gene
peptides are detected by their interaction with an anti-
fingerprint gene product-specific antibody.

        For example, antibodies, or fragments of antibodies,
such as those described, above, in Section 5.6, useful in the
30 present invention can be used to quantitatively or
qualitatively detect the presence of wild type or mutant
fingerprint gene peptides. This can be accomplished, for
example, by immunofluorescence techniques employing a
fluorescently labeled antibody (see below, this Section,)
35 coupled with light microscopic, flow cytometric, or
fluorimetric detection. Such techniques are especially
preferred if the fingerprint gene peptides are expressed on

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the cell surface, such as, for example, is the case with the 10 gene product, the 200 gene product and the transmembrane form of 103 gene product. Thus, the techniques described herein can be used to detect specific cells, within a
5 population of cells, which express the fingerprint gene product of interest.

The antibodies (or fragments thereof) useful in the present invention can, additionally, be employed histologically, as in immunofluorescence or immunoelectron
10 microscopy, for _in situ_ detection of fingerprint gene peptides. _In situ_ detection can be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody of the present invention. The antibody (or fragment) is preferably applied by overlaying the labeled
15 antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the fingerprint gene peptides, but also their distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that
20 any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such _in situ_ detection.

Immunoassays for wild type or mutant fingerprint gene peptides typically comprise incubating a biological sample,
25 such as a biological fluid, a tissue extract, freshly harvested cells, or cells which have been incubated in tissue culture, in the presence of a detectably labeled antibody capable of identifying fingerprint gene peptides, and detecting the bound antibody by any of a number of techniques
30 well-known in the art.

The biological sample can be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The
35 support can then be washed with suitable buffers followed by treatment with the detectably labeled fingerprint gene-specific antibody. The solid phase support can then be

washed with the buffer a second time to remove unbound
antibody. The amount of bound label on solid support can
then be detected by conventional means.

By "solid phase support or carrier" is intended any
5 support capable of binding an antigen or an antibody. Well-
known supports or carriers include glass, polystyrene,
polypropylene, polyethylene, dextran, nylon, amylases,
natural and modified celluloses, polyacrylamides, gabbros,
and magnetite. The nature of the carrier can be either
10 soluble to some extent or insoluble for the purposes of the
present invention. The support material can have virtually
any possible structural configuration so long as the coupled
molecule is capable of binding to an antigen or antibody.
Thus, the support configuration can be spherical, as in a
15 bead, or cylindrical, as in the inside surface of a test
tube, or the external surface of a rod. Alternatively, the
surface can be flat such as a sheet, test strip, etc.
Preferred supports include polystyrene beads. Those skilled
in the art will know many other suitable carriers for binding
20 antibody or antigen, or will be able to ascertain the same by
use of routine experimentation.

The binding activity of a given lot of anti-wild type or
mutant fingerprint gene product antibody can be determined
according to well known methods. Those skilled in the art
25 will be able to determine operative and optimal assay
conditions for each determination by employing routine
experimentation.

One of the ways in which the fingerprint gene peptide-
specific antibody can be detectably labeled is by linking the
30 same to an enzyme and use in an enzyme immunoassay (EIA)
(Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)",
1978, Diagnostic Horizons 2:1-7, Microbiological Associates
Quarterly Publication, Walkersville, MD); Voller, A. et al.,
1978, J. Clin. Pathol. 31:507-520; Butler, J.E., 1981, Meth.
35 Enzymol. 73:482-523; Maggio, E. (ed.), 1980, ENZYME
IMMUNOASSAY, CRC Press, Boca Raton, FL; Ishikawa, E. et al.,
(eds.), 1981, ENZYME IMMUNOASSAY, Kgaku Shoin, Tokyo). The

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enzyme which is bound to the antibody will react with an
appropriate substrate, preferably a chromogenic substrate, in
such a manner as to produce a chemical moiety which can be
detected, for example, by spectrophotometric, fluorimetric or
5 by visual means. Enzymes which can be used to detectably
label the antibody include, but are not limited to, malate
dehydrogenase, staphylococcal nuclease, delta-5-steroid
isomerase, yeast alcohol dehydrogenase, alpha-
glycerophosphate, dehydrogenase, triose phosphate isomerase,
10 horseradish peroxidase, alkaline phosphatase, asparaginase,
glucose oxidase, beta-galactosidase, ribonuclease, urease,
catalase, glucose-6-phosphate dehydrogenase, glucoamylase and
acetylcholinesterase. The detection can be accomplished by
colorimetric methods which employ a chromogenic substrate for
15 the enzyme. Detection can also be accomplished by visual
comparison of the extent of enzymatic reaction of a substrate
in comparison with similarly prepared standards.

Detection can also be accomplished using any of a
variety of other immunoassays. For example, by radioactively
20 labeling the antibodies or antibody fragments, it is possible
to detect fingerprint gene wild type or mutant peptides
through the use of a radioimmunoassay (RIA) (see, for
example, Weintraub, B., Principles of Radioimmunoassays,
Seventh Training Course on Radioligand Assay Techniques, The
25 Endocrine Society, March, 1986, which is incorporated by
reference herein). The radioactive isotope can be detected
by such means as the use of a gamma counter or a
scintillation counter or by autoradiography.

It is also possible to label the antibody with a
30 fluorescent compound. When the fluorescently labeled
antibody is exposed to light of the proper wavelength, its
presence can then be detected due to fluorescence. Among the
most commonly used fluorescent labeling compounds are
fluorescein isothiocyanate, rhodamine, phycoerythrin,
35 phycocyanin, allophycocyanin, o-phthaldehyde and
fluorescamine.

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The antibody can also be detectably labeled using
fluorescence emitting metals such as ^{152}Eu, or others of the
lanthanide series. These metals can be attached to the
antibody using such metal chelating groups as
5 diethylenetriaminepentacetic acid (DTPA) or
ethylenediaminetetraacetic acid (EDTA).

The antibody also can be detectably labeled by coupling
it to a chemiluminescent compound. The presence of the
chemiluminescent-tagged antibody is then determined by
10 detecting the presence of luminescence that arises during the
course of a chemical reaction. Examples of particularly
useful chemiluminescent labeling compounds are luminol,
isoluminol, theromatic acridinium ester, imidazole,
acridinium salt and oxalate ester.

15   Likewise, a bioluminescent compound can be used to label
the antibody of the present invention. Bioluminescence is a
type of chemiluminescence found in biological systems in,
which a catalytic protein increases the efficiency of the
chemiluminescent reaction. The presence of a bioluminescent
20 protein is determined by detecting the presence of
luminescence. Important bioluminescent compounds for
purposes of labeling are luciferin, luciferase and aequorin.

    6.   <u>EXAMPLE: IDENTIFICATION AND CHARACTERIZATION OF A TH2-</u>
                              <u>ENRICHED GENE</u>
25
    In the Example presented in this Section, the transgenic
T cell paradigm described, above, in Section 5.1.1.1, , was
utilized to identify a gene, designated herein as the 102
gene, which is expressed in TH2 cells. The identified gene
30 is present in TH2 cells at a much higher level than in TH1
cells. Thus, the Example presented herein demonstrates the
usefulness of the paradigm approach of the invention for the
identification of genes that are differentially expressed in
TH cell subpopulations.

35

6.1. MATERIALS AND METHODS

Transgenic mice: Naive CD4⁺ cells were obtained from the spleens and/or lymph nodes of unprimed transgenic mouse strains harboring a T cell receptor (TCR) recognizing
5 ovalbumin (Murphy et al., 1990, Science 250:1720).

Ova-specific transgenic T cells: Suspensions of ova-specific T cells were co-cultured with stimulatory peptide antigen and antigen presenting cells essentially as described
10 in Murphy et al. (Murphy et al., 1990, Science 250:1720). Briefly, 2-4 x 10⁶ T cells were incubated with approximately twice as many TA3 antigen presenting cells in the presence of 0.3µM Ova peptide. TH1 cultures contained approximately 10 ng/ml recombinant mIL-12. Conversely, TH2 cells received IL-
15 4 (1000u/ml). Cultures were harvested at various time points after initiation of culture. T cells were purified of TA3 cells using anti-CD4 coated magnetic beads (Dynal, Inc.). T cells were pelleted by gentle centrifugation and lysed in the appropriate volume of RNAzol™ (Tel-Test, Friendswood, TX).
20

Tissue collection and RNA isolation: Cells were quick frozen on dry ice. Samples were then homogenized together with a mortar and pestle under liquid nitrogen.

Total cellular RNA was extracted from tissue with either
25 RNAzol™ or RNAzolB™ (Tel-Test, Friendswood, TX), according to the manufacturer's instructions. Briefly, the tissue was solubilized in an appropriate amount of RNAzol™ or RNAzolB™, and RNA was extracted by the addition of 1/10 v/v chloroform to the solubilized sample followed by vigorous shaking for
30 approximately 15 seconds. The mixture was then centrifuged for 15 minutes at 12,000g and the aqueous phase was removed to a fresh tube. RNA was precipitated with isopropanol. The resultant RNA pellet was dissolved in water and re-extracted with an equal volume of chloroform to remove any remaining
35 phenol. The extracted volume was precipitated with 2 volumes of ethanol in the presence of 150mM sodium acetate. The

precipitated RNA was dissolved in water and the concentration determined spectroscopically (A_{260}).

Differential display: Total cellular RNA (10-50μg)
5 was treated with 20 Units DNase I (Boehringer Mannheim, Germany) in the presence of 40 Units ribonuclease inhibitor (Boehringer Mannheim, Germany). After extraction with phenol/chloroform and ethanol precipitation, the RNA was dissolved in DEPC (diethyl pyrocarbonate)-treated water.
10   Differential mRNA display was carried out as described, above, in Section 5.1.1.2. RNA (0.4-2μg) was reverse-transcribed using Superscript reverse transcriptase (GIBCO/BRL). The cDNAs were then amplified by PCR on a Perkin-Elmer 9600 thermal cycler. The reaction mixtures
15 (20μl) included arbitrary decanucleotides and one of twelve possible $T_{11}VN$ sequences, wherein V represents either dG, dC, or dA, and N represents either dG, dT, dA, or dC. Parameters for the 40 cycle PCR were as follows: Hold 94° C. 2 minutes; Cycle 94° C. 15 seconds, 40° C. 2 minutes; Ramp to 72° 30
20 seconds; Hold 72° C. 5 minutes; Hold 4° C.
        Radiolabelled PCR amplification products were analyzed by electrophoresis on 6% denaturing polyacrylamide gels.

Reamplification and subcloning: PCR bands of interest
25 were recovered from sequencing gels and reamplified.
        Briefly, autoradiograms were aligned with the dried gel, and the region containing the bands of interest was excised with a scalpel. The excised gel fragment was eluted by soaking in 100μl TE (Tris-EDTA) buffer at approximately 100°
30 C. for 15 minutes. The gel slice was then pelleted by brief centrifugation and the supernatant was transferred to a new microcentrifuge tube. DNA was combined with ethanol in the presence of 100mM Sodium acetate and 30μg glycogen (Boerhinger Mannhein, Germany) and precipitated on dry ice
35 for approximately 10 minutes. Samples were centrifuged for 10 minutes and pellets were washed with 80% ethanol. Pellets were resuspended in 10μl distilled water.

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5μl of the eluted DNA were reamplified in a 100μl
reaction containing: standard Cetus Taq polymerase buffer,
20μM dNTPs, 1μM of each of the oligonucleotide primers used
in the initial generation of the amplified DNA.   Cycling
5 conditions used were the same as the initial conditions used
to generate the amplified band, as described above.   One-half
of the amplification reaction was run on a 2% agarose gel and
eluted using DE-81 paper (Whatman Paper, Ltd., England) as
described in Sambrook et al., _supra_.   Recovered fragments
10 were ligated into the cloning vector pCR^{tm}II (Invitrogen,
Inc., San Diego CA) and transformed into competent _E. coli_
strain DH5α (Gibco/BRL, Gaithersburg, MD).   Colonies were
grown on LB-agar plates containing ampicillin (100μg/ml) and
X-gal (40μg/ml) to permit blue/white selection.
15

Sequence analysis: After subcloning, reamplified cDNA
fragments were sequenced on an Applied Biosystems Automated
Sequencer (Applied Biosystems, Inc. Seattle, WA).   Sequence
was obtained from four or more independent transformants
20 containing the same insert.   The nucleotide sequence shown
herein represents either the consensus of the information
obtained from the four sequences, or the sequence obtained
from a representative clone, as indicated.   Such primary
sequence data was edited and trimmed of vector sequences and
25 highly repetitive sequences and used to search Genbank
databases using the BLAST (Altschul, S.F. et al., 1990, J.
Mol. Biol. 215:403-410) program.

Northern analysis: RNA samples were electrophoresed in
30 a denaturing agarose gel containing 1-1.5% agarose (SeaKem™
LE, FMC BioProducts, Rockland, ME) containing 6.3%
formaldehyde.   Samples containing 5-20μg of total RNA were
mixed with denaturing loading solution (72% deionized
formamide and bromophenol blue) and heated to 70°C. for 5
35 minutes.   Samples were placed on ice and immediately loaded
onto gels.   Gels were run in 1x MOPS buffer (100mM MOPS, 25mM
sodium acetate, 5mM EDTA).   After electrophoresis, the gels

were stained with ethidium bromide and visualized with
ultraviolet light.

After completion of electrophoresis, gels were soaked in
50mM sodium hydroxide with gentle agitation for approximately
5   30 minutes to lightly cleave RNA. Gels were rinsed twice in
water and then neutralized by soaking in 0.1M Tris-HCl (pH
7.5) for approximately 30 minutes. Gels were briefly
equilibrated with 20x SSC (3M sodium chloride, 0.3M sodium
citrate) and then transferred to nylon membranes such as
10 Hybond™,-N, (Amersham, Inc., Arlington Heights, IL) or Zeta-
Probe (Bio-Rad, Inc., Hercules, CA) overnight in 20x SSC.
Membranes containing transferred RNA were baked at 80°C. for
2 hours to immobilize the RNA.

DNA fragments to be used as probes were of various sizes
15 and were labeled using a random hexamer labeling technique.
Briefly, 25ng of a purified DNA fragment was used to generate
each probe. Fragments were added to a 20μl random
hexanucleotide labeling reaction (Boehringer Mannhein, Inc.,
Indianapolis, IN) containing random hexamers and a mix of the
20 nucleotides dCTP, dGTP, and dTTP (at a final concentration of
25μM each). The reaction mix was heat-denatured at 100°C.
for 10 minutes and then chilled on ice. 5μl of α-^{32}P-dATP
(50μCi; Amersham, Inc., Arlington Heights, IL) and Klenow DNA
polymerase (2 units; Boehringer Mannheim, Inc., Indianapolis,
25 IN) were added. Reactions were incubated at 37° for 30
minutes. Following incubation, 30μl water was added to the
labeling reaction and unincorporated nucleotides were removed
by passing the reactions through a BioSpin-6™ chromatography
column (Bio-Rad, Inc., Hercules, CA). Specific incorporation
30 was determined using a scintillation counter. 1-5 x10^6 cpm
were used per ml hybridization mixture.

Nylon membranes containing immobilized RNA were
prehybridized according to manufacturer's instructions.
Radiolabelled probes were heat denatured at 70°C. in 50%
35 deionized formamide for 10 minutes and ten added to the
hybridization mixture (containing 50% formamide, 10% dextran
sulfate, 0.1% SDS, 100μg/ml sheared salmon sperm DNA, 5x SSC,

5x Denhardt's solution, 30mM Tris-HCl (pH 8.5), 50mM NaPO₄
(pH 6.5). Hybridizations were carried out at 42°C.
overnight. Nylon membranes were then bathed for 2 minutes in
a wash solution of 0.2x SSC and 0.1% SDS at room temperature
5 to remove most of the remaining hybridization solution. The
membranes were then bathed twice in fresh 42°C. preheated
wash solution for 20 minutes. Filters were covered in
plastic wrap and exposed to autoradiographic film to
visualize results.
10

6.2. RESULTS

A transgenic T cell paradigm (as described, above, in
Section 6.1) was utilized to identify genes which are
differentially expressed between TH1 and TH2 cells.
15   RNA samples were isolated from TH1 and TH2 cell
populations after either secondary or tertiary antigen
stimulation. The samples were then analyzed via differential
display techniques. FIG. 1 shows amplified fragments
obtained from these samples, with the arrow indicating a PCR
20 product, designated band 102, which was judged to represent a
cDNA derived from RNA produced by a gene which is expressed
at a higher level in TH2 cell subpopulations, relative to TH1
cell subpopulations. The gene corresponding to band 102 is
referred to herein as the 102 gene.
25   The amplified band 102 cDNA was recovered, reamplified,
subcloned into a cloning vector and sequenced, as described,
above, in Section 6.1. The nucleotide sequence (SEQ ID NO:1)
of a representative band 102 clone, specifically, clone
102.1, is shown in FIG. 2.
30   A BLAST (Altschul, S.F. et al., 1990, J. Mol. Biol.
215:403-410) database search with this consensus sequence
resulted in an alignment with 98% identity to the mouse
Granzyme A, or Hanukah factor, gene, (Masson, D. et al.,
1986, FEBS Lett. 208:84-88; Masson, D. et al., 1986, EMBO J.
35 5:1595-1600; Gershenfeld, H.K. and Weissman, I.L., 1986,
Science 232:854-858), which encodes a trypsin-like serine
protease. The human homolog of this gene is also known

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(Gershenfeld, H.K. et al., 1988, Proc. Natl. Acad. Sci. USA
85:1184-1188).

To confirm the gene's putative differential regulation,
amplified band 102 cDNA was used to probe Northern RNA blots
5 containing RNA samples from TH1 and TH2 cell lines, and from
spleen and thymus tissue. FIG. 3 shows the results of one
such Northern blot analysis, in which the steady state level
of message for 102 gene mRNA are significantly increased in
RNA samples derived from stimulated TH2 versus TH1 samples.
10 Further, the positive signals in both thymus and spleen RNA
samples supports the indication that the 102 gene product is
involved in some aspect of T cell function.   Thus, the
Northern analysis confirmed the putative differential TH2
regulation which had been suggested by the differential
15 display result.

Therefore, by utilizing the transgenic T cell paradigm
described in this Section and in Section 5.1.1.1, above, a
TH2 differentially regulated gene, designated here the 102
gene, and corresponding to the mouse Granzyme A/Hanukah
20 factor gene, has been identified, thereby corroborating the
usefulness of such paradigms in identifying genes expressed
preferentially in T helper cell subpopulations such as TH1 or
TH2 cell populations.

Further, while the gene identified here had previously
25 been found to be expressed in natural killer T cells and,
further, had been reported to be expressed in a fraction of
CD4· cells (Fruth, U. et a;., 1988, Eur. J. Imm. 18:773-781;
Liu, C.C. et al., 1989, J. Exp. Med. 170:2105-2118), the
results described herein represent the first instance in
30 which a TH cell subpopulation role for this gene has been
found. Prior to this study, the gene had been reported to be
expressed in T cells in a variety of situations, including
TH1 cell subpopulation- and TH2 cell subpopulation-related
disorders. For example, Granzyme A/Hanukah factor expression
35 has been reported in allograft rejection (Muller, C. et al.,
1988, J. Exp. Med. 167:1124-1136) and autoimmune diseases
(Ojcius, D.M. and Young, D.E., 1990, Cancer Cells 2:138-145;

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Young, L.H.Y. et al., 1992, Am. J. Path. <u>140</u>:1261-1268),
which are TH1 cell subpopulation-related disorders, and also
in Leishmania infection susceptible mice (Moll, H. et al.,
1991, Inf. and Imm. <u>59</u>:4701-4705) and in leprosy lesions
5 (Ebnet, K. et al., 1991, Int. Imm. <u>3</u>:9-19; Cooper, C.L. et
al., 1989, J. Exp. Med. <u>169</u>:1565-1581), which are both TH2
cell subpopulation-related disorders.

The differential TH2-like expression demonstrated here
represents, therefore, the first molecular evidence clearly
10 indicating a more primary role for the gene product in TH2
versus TH1 cell subpopulations.

7. EXAMPLE: IDENTIFICATION AND CHARACTERIZATION OF A TH2-SPECIFIC GENE

15   In the Example presented in this Section, the transgenic
T cell paradigm, described, above, in Sections 5.1.1.1 and 6,
was utilized to identify a gene which is differentially
expressed in TH2 cells. Specifically, this gene is present
in TH2 cells while being completely absent from TH1 cells.
20 The gene, which corresponds to a gene known, alternatively,
as ST-2, T1 and Fit-1, does not appear to be expressed in any
other assayed cell type or tissue, and is demonstrated here
for the first time to encode a marker which is, in vivo,
completely TH2-specific. The 103 gene encodes a cell surface
25 protein, the potential significance of which is discussed
herein.

7.1. MATERIALS AND METHODS

RT-PCR analysis: Quantitative RT-PCR was performed as
30 follows. 1-2μg of total RNA, prepared as described, above,
in Section 6.1, was reverse transcribed with oligo dT$_{(12-18)}$
primers and Superscript™ RNAase H⁻ reverse transcriptase
(Gibco-BRL, Gaithersburg, MD). Briefly, RNA was combined
with 1μl oligo dT (500μg/ml) in a total volume of 11μl. The
35 mixture was heated to 70° C for 10 minutes and chilled on
ice. After a brief centrifugation, RNA was reverse

transcribed for 1 hour. Aliquots of the first strand cDNA
were stored at -20° C. until just prior to use.

Expression levels were determined by PCR amplification
of serial dilutions of first strand cDNA. In this procedure,
5 cDNA is serially diluted in water. The dilutions are then
batch amplified by PCR using sequence-specific primers. All
PCR reactions are amplified under identical conditions.
Therefore, the amount of product generated should reflect the
amount of sequence template which was initially present. 5-
10 10 fold dilutions of cDNA were used and enough dilutions were
used such that the amount of product subsequently produced
ranged from clearly visible, by UV illumination of ethidium
bromide-stained gels, to below detection levels. The method
described herein can distinguish 10-fold differences in
15 expression levels.

Primers were designed for the amplification of the
sequenced amplified bands, which were chosen using the
program OLIGO (National Biosciences, Plymouth, MN). Primer
sequences used in this assay were as follows: and 103 sense
20 primer, 5'-TTGCCATAGAGAGACCTC-3' (SEQ ID NO:18); band 103
antisense primer, 5'-TGCTGTCCAATTATACAGG-3' (SEQ ID NO:19);
murine gamma actin sense primer, 5'-GAACACGGCATTGTCACTAACT-3'
(SEQ ID NO:20); murine gamma actin antisense primer, 5'-
CCTCATAGATGGGCACTGTGT-3' (SEQ ID NO:21).
25   All quantitative PCR reactions were carried out in a
9600 Perkin-Elmer PCR machine (Perkin-Elmer). Generally,
amplification conditions were as follows: 30-40 cycles
consisting of a 95° C denaturation for 30 seconds, 50-60° C
annealing for 30 seconds, and 72° C extension for 1 minute.
30 Following cycling, reactions were extended for 10 minutes at
72° C.

RNase Protection Assays: RNAse protection assays were
performed according to manufacturer's instructions, using a
35 kit purchased from Ambion, Inc. RNA probes derived from
GenBank Accession No. Y07519 were utilized in the RNAse
protection assays. These probes were also generated

according to manufacturer's instructions, using a kit
purchased from Ambion, Inc. The sequence of these RNA probes
corresponds to the 5' end of the gene, and includes both
coding and 5' untranslated sequences.

5

    Anti CD-3 stimulation: Conditions were as described,
below, in Section 8.1.

    Other procedures: All other cell sample collection, RNA
10 isolation, differential display, sequence analysis, and
Northern procedures performed in the experiments described in
this Example were as described, above, in Section 6.1.

                    7.2. RESULTS
15   A differential display analysis of RNA isolated from TH1
and TH2 cell samples obtained from a transgenic T cell
paradigm study as described, above, in Section 6.1.
Specifically, TH cells were obtained from transgenic mice
harboring a T cell receptor recognizing ovalbumin (Murphy et
20 al., 1990, Science 250:1720) were stimulated three times, and
RNA was obtained from TH1 and TH2 cells. Differential
display analysis of the RNA samples resulted in the
identification of a TH2 differentially expressed band,
designated and referred to herein as band 103. The gene
25 corresponding to band 103 is referred to herein as the 103
gene.
    103 gene cDNA was isolated, amplified and subcloned, and
nucleotide sequence (SEQ ID NO:2) was obtained, as shown in
FIG. 4A. A database search revealed that the nucleotide
30 sequence of band 103 resulted in an alignment with 98%
identity to the mouse form of a gene known, alternatively, as
the ST-2, T1 or Fit-1 gene (Klemenz, R. et al., 1989, Proc.
Natl. Acad. Sci. USA 86:5708-5712; Tominaga, S., 1989, FEBS
Lett. 258:301-301; Werenskiold, A.K. et al., 1989, Mol. Cell.
35 Biol.9:5207-5214; Werenskiold, A.K., 1992, Eur. J. Biochem.
204:1041-1047; Yanagisawa, K. et al., 1993, FEBS Lett.
318:83-87; Bergers, G. et al., 1994, EMBO J. 13:1176-1188).

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The 103 gene encodes, possibly via alternatively spliced
transcripts, transmembrane and soluble forms of proteins
which belong to the immunoglobulin superfamily. The soluble
form of the protein shows a high level of similarity to the
5 extracellular portion of the mouse interleukin-1 receptor
type 1 (IL-1R1) and interleukin-1 receptor type 2 (IL-1R2;
which lacks a cytoplasmic domain), while the transmembrane
portion (termed ST2L) bears a high resemblance to the entire
IL-1R1 sequence and to the extracellular IL-1R2 sequences.
10 Further, the 103 gene appears to be tightly linked to the
interleukin 1 receptor-type 1 locus (McMahan, C.J. et al.,
1991, EMBO J. 10:2821-2832; Tominaga, S. et al., 1991,
Biochem. Biophys. Acta. 1090:1-8). Additionally, the human
103 gene homolog has also been reported (Tominaga, S. et al.,
15 1992, Biochem. Biophys. Acta. 1171:215-218). FIG. 4B
illustrates the 103 gene transmembrane and soluble forms of
protein, and shows their relationship to the IL-1R1 protein
sequence.

A quantitative RT-PCR analysis (FIG. 5) of RNA obtained
20 from cells of a TH1 and TH2 cells, generated as described
above, 24 hours after tertiary antigen stimulation not only
confirmed the putative TH2 differential expression of the
gene, but, revealed that the expression of the 103 gene
appears to be TH2 specific, i.e., the sensitive RT-PCR study
25 detected no 103 gene message in the TH1 RNA sample.

The TH2 specificity of the 103 gene was further
confirmed by a Northern analysis of several representative TH
cell lines. Specifically, three TH2 clones (CDC25, D10.G4,
DAX) and three TH1 clones (AE7.A, Dorris, D1.1) were utilized
30 and RNA samples were isolated from either unstimulated cells
or from cells which had been stimulated for 6 hours with
plate-bound anti-CD3 antibody. The samples were probed with
band 103 sequences, as shown in FIG. 6. While 103 gene RNA
is present in RNA obtained from both unstimulated and
35 stimulated cells of each of the TH2 cell lines, 103 gene RNA
is completely absent from all of the samples obtained from
either stimulated or unstimulated TH1 cells. As the RT-PCR

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analysis described above first demonstrated, the 103 gene
appears to be TH2 specific, with no detectable TH1-derived
signal being present.

The data presented in FIG. 7 represent an additional
5 Northern analysis in which 103 gene expression was assayed in
TH cell clones (lanes 1-5) and in murine tissues (lanes 6-
10). In addition to corroborating the expression of 103 gene
RNA in both stimulated and unstimulated TH2 cells, the data
presented here demonstrate that 103 gene expression appears
10 to be negative in each of the tissues (i.e., brain, heart,
lung, spleen, and liver) tested.

FIG. 8 illustrates an RNAse protection assay which
demonstrates two points regarding 103 gene regulation.
First, this analysis of TH cell clones confirms the TH2-
15 specific results described, above. Specifically, the results
of this study demonstrate by RNase protection, that 103 gene
mRNA is absent from the TH1 clone AE7, but is present in the
TH2 clone D10.G4.

Second, RNAse protection revealed that alternate forms
20 of 103 gene transcripts are produced upon stimulation of TH2
clones. Specifically, within 6 hours of anti-CD3
stimulation, two additional forms of 103 gene transcript
appear in TH2 clones. These additional 103 gene transcript
forms represent, one, a transcript encoding a shortened,
25 secreted, soluble form of the band 103 gene product, and,
two, a smaller, termed mini, transcript which encodes a yet
shorter form of the gene product. Thus, it appears that,
while the 103 gene transcript encoding the transmembrane gene
product is expressed in both unstimulated and stimulated TH2
30 cells, the two shorter forms of transcript are expressed in a
TH2-specific inducible manner. Further, while the 103 gene
transcript encoding the transmembrane product are expressed
in both stimulated and unstimulated TH2 cells, the level of
this transcript present in stimulated is lower, i.e., is
35 downregulated. Thus, the lower level of transmembrane
product and higher level of secreted 103 gene product can act

synergistically to dampen some stimulation-induced signal transduction event.

Additionally, it should be noted that the results presented herein represent the first time the mini form of
5 103 gene transcript, which can encode a shorter version of the soluble form of 103 gene product, has been observed.

To summarize, while 103 gene expression in T helper cell lines had previously been reported (Tominaga, S. et al., 1992, Biochem. Biophys. Acta. 1171:215-218), the TH
10 paradigm/differential display techniques utilized here have demonstrated, for the first time, that the 103 gene encodes a TH2 cell subpopulation-specific surface marker. In fact, the results described in this Example demonstrate that the first identification of any in vivo TH cell subpopulation-specific
15 cellular marker.

Given its status as both a TH2 cell subpopulation-specific marker and cell surface protein, the full length 103 gene product can be utilized in a variety of methods to modulate TH cell subpopulation-related disorders and/or to
20 identify compounds which exhibit such modulatory capability. The truncated forms of the 103 gene products can, additionally, be used as part of these methods. Modulatory methods are described, above, in Section 5.9, while strategies for the identification of modulatory compounds are
25 described, above, in Section 5.8.

8.   EXAMPLE: IDENTIFICATION OF NOVEL TH CELL SUBPOPULATION DIFFERENTIALLY EXPRESSED GENES

In the Example presented in this Section, novel gene
30 sequences representing genes which are differentially expressed in TH cell subpopulations and/or during the differentiation of such subpopulations are described.

8.1.   MATERIALS AND METHODS

35 T cell clone paradigm: T cell clone paradigm searches were conducted as described, above, in Section 5.1.1.1. Specifically, the TH cell clone paradigms used three

different clones: D10.G4 (TH2), AE7 (TH1) and D1.1 (TH1).
Prior to stimulation, cell cultures were enriched for live
cells by centrifugation through a Ficoll gradient. Recovered
cells were counted and their viability was examined using
5 trypan blue exclusion. Cells were replated into either T25
or T75 flasks at approximately 5 x 10^6 cells in 5 mls or 1.5
x 10^6 cells in 10 mls of culture medium, respectively.

Coating was performed, generally, according to Current
Protocols in Immunology, 1992, Coligan, J.E. et al., John
10 Wiley & Sons, NY, pp 3.12.4-3.12.6). Specifically, prior to
plating, the flasks were coated with anti-CD3-ε antibodies
(hybridoma supernatant from the 145-C11 hybridoma; Parmingen,
Inc., San Diego CA). For coating, antibodies were resuspended
in PBS at 1-2 μg/ml at a volume sufficient to coat the bottom
15 of the flasks. Coating solution was incubated on the flasks
for at least one hour at 37° C.

After incubation, the antibody coating solution was
removed by aspiration and cells were immediately added.
Flasks were placed in a 37° C incubator for 6 hours. Cells
20 were harvested by, for example, removal of supernatant from
the culture, followed by direct lysing of cells by addition
of RNAzol™ solution. cDNA was produced as described below.

cDNA isolation: RNA was harvested from cells using
techniques described, above, in Section 6.1. mRNA was
25 purified directly, using a QuickPrep™ mRNA Purification Kit
(Pharmacia) according to manufacturer's instructions.

The TH1 cDNA library was constructed using a Gibco BRL
SuperScript™ Lambda System Kit, according to manufacturer's
instructions. Briefly, 4.5 μg of purified mRNA was used as
30 starting material for the synthesis of poly A-primed first
strand cDNA containing a Not-1 cloning site. Second strand
cDNA synthesis was performed with RNAse H treatment followed
by random priming. Sal-1 adaptors were ligated to the 5' end
of the resulting double-stranded cDNA. The ligated cDNA was
35 digested with Not-1 and size fractionated. Fractions
containing cDNAs within the size range of 0.5 to 8.0 kb in
length were cloned into Sal-1/Not-1 λZipLox™ arms.

Recombinant phage was then packaged using the Stratagene
Gigapack™ II Packaging Extracts Kit, according to
manufacturer's instructions. E. coli strain Y 1090(ZL)™
(Gibco BRL) cells were transformed with packaged recombinant
5 phage and plated at a density of 50,000 pfu per 150mm dish.
Plaques were screened by hybridization to a radiolabelled
probe generated from a subcloned band 200 cDNA fragment.
Excision of cDNA inserts from lambda clones and introduction
of the recombinant plasmid DNA into E. coli DH10B(ZIP)™
10 (Gibco BRL) was performed according to manufacturer's
instructions.

For isolation of 200 gene cDNAs, the cDNA library was
screened with a probe generated by labeling the entire
sequence of the band 200 subclone O, which was constructed
15 using amplified DNA obtained from the differential display
analysis. The band 200 sequence was excised from the pCRII
Cloning Vector™ (Invitrogen) by digestion with EcoRI.
Approximately 1/100,000 cDNA library plaques were scored as
positive when screened with this probe. Several clones,
20 including 200-P and 200AF, were chosen for further study.

The cDNA library described above was also used to
isolate 54 gene cDNA clones. For screening, the entire
excised band 54 insert was used as a probe.

Other procedures: All transgenic T cell manipulations,
25 cell sample collection, additional RNA isolation,
differential display, sequence analysis, and Northern
procedures performed in the experiments described in this
Example were as described, above, in Section 6.1.

30   8.2. RESULTS

Transgenic T cell paradigm and T cell clone paradigm
searches were conducted to identify gene sequences which
represent genes differentially expressed within and/or among
TH cell subpopulations and/or during the differentiation of
35 such subpopulations. Described herein are several novel
genes which have been identified via these paradigm searches.
Specifically, the genes described herein have been designated

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the 10, 54, 57, 105, 106, 161 and 200 genes. A summary of
the differential expression characteristics of the novel gene
sequences described herein is presented in Table 1, above.

The band 10 and 57 have been identified as TH inducible
5 gene sequences. That is, the expression of such genes in
unstimulated TH cells is either undetectable or is detectable
at extremely low levels, but is upregulated in both
stimulated TH1 and TH2 cells. In fact, the 10 gene
expression is detectable as early as 6 hours post
10 stimulation. Thus, such gene products can be involved in the
activation of TH cells and/or can be involved in the
maintenance of mature TH cell function, in a non-TH cell
subpopulation-specific manner.

FIG. 9 depicts the nucleotide sequence (SEQ ID NO:3) of
15 the 10 gene coding region and the derived amino acid sequence
of the 10 gene product (SEQ ID NO:10). While database
searches reveal that the 10 gene sequence is novel, that is,
has not previously been reported in the databases, an
analysis of the portion of the 10 gene corresponding to the
20 band 10 nucleotide sequence (the underlined portion of the
nucleotide sequence of FIG. 9) shows, as depicted in FIG.
10A-C, a high similarity to a specific class of known gene
products. Specifically, as the hydrophilicity plots of FIG.
10A-C show, the 10 gene product appears to encode a protein
25 having a seven transmembrane domain sequence motif.
Interestingly, the gene products belonging to this class of
protein tend to represent G protein-coupled receptor
molecules. (See, e.g., Larhammar, D. et al., 1992, J. Biol.
Chem. 267: 10935-10938; Law, S.F. et al., 1991, J. Biol.
30 Chem. 266: 17885-17997.) Thus, the TH inducible expression
of the 10 gene coupled with the predicted protein structure
of its gene product, suggests that the 10 gene product is
involved in a signal transduction event important to the
differentiation of mature TH cells.

35   Additionally, as the map shown in FIG. 11 indicates, the
chromosomal location of the murine 10 gene has been
identified. The 10 gene locus is located on Chromosome 12,

is closely linked to a class of genes encoding T cell
autoantigens, and additionally, maps near the Ig heavy chain
gene locus.

     The nucleotide sequence (SEQ ID NO:4) of a
5 representative band 57 clone is depicted in FIG. 12. The
gene corresponding to band 57 is the 57 gene. The 57 gene
appears to be a novel gene sequence in that it does not
appear within the published databases. No homology to known
peptide domains has, thus far, been identified.

10   As shown in Table 1, above, the genes 105, 106 and 200
are each expressed at a higher level within the TH1 cell
subpopulation, as revealed by the TH1 differential appearance
of amplified bands 105, 106 and 200. Nucleotide sequences
contained within bands 105 and 106 are depicted in FIGS. 13
15 (SEQ ID NO:5) and 14 (SEQ ID NO:6), respectively. As
discussed below, the sequence of the murine 200 gene is
depicted in FIG. 17 (SEQ ID NO:8). None of these sequences
appear within published databases. Given the TH1-specific
expression pattern each of these sequences exhibits, the
20 genes and their gene products can potentially be used as
treatments for TH1-related disorders, as diagnostics for such
disorders, and/or as part of methods for the identification
of compounds capable of ameliorating TH1-related disorders.

     The 161 gene appears to be TH cell subset specific.
25 That is, 161 gene expression has been observed in either TH1
cells or in TH2 cells, but its expression has never been
observed, simultaneously, in both TH1 and TH2 cell
subpopulations. The details of the 161 gene differential
expression pattern are currently being elucidated. It is
30 possible that 161 gene expression is indicative of the
presence of yet another TH cell subpopulation, in addition to
TH1, TH2 and TH0 cell subpopulations.

     FIG. 15 presents the band 161 nucleotide sequence.
While the 161 gene appears to be a novel sequence, it bears a
35 distinct level of similarity to a set of gene sequences (SEQ
ID NOS:13-17) in published databases, as shown in FIG. 16.

Interestingly, the genes within this group each contain alpha-interferon responsive promoters.

Band 200 was utilized as a probe to identify and isolate murine 200 gene cDNA clones, including clones designated
5 200-P, 200-AF and 200-O, which have been deposited with the NRRL, as summarized in Section 10, below. The cDNA clones were characterized, yielding the full length nucleotide sequence (SEQ ID NO:8) of the murine 200 gene coding region, as shown in FIG. 17. FIG. 17 also depicts the murine 200
10 gene product derived amino acid sequence (SEQ ID NO:10).
Database searches reveal that the 200 gene product is a novel receptor which contains an extracellular Ig domain, thus placing it within the Ig receptor superfamily. The cloning and characterization of the 200 gene human homolog is
15 described in the Example presented in Section 9, below.

The results of a murine 200 gene mRNA Northern blot analysis are shown in FIG. 18. The data depicted in FIG. 18 demonstrates, first, that the 200 gene produces a transcript of approximately 1.2 kb in length, and, second, illustrates
20 the TH1 specificity of 200 gene expression

For the study, three TH1 clones (D1.1, Dorris, AE7) and three TH2 clones (D10G.4, DAX, CDC25) were utilized, and RNA samples were isolated from either unstimulated cells (-) or cells which had been stimulated for 6 hours with plate-bound
25 anti-CD3 antibody (+). The samples were probed with 200 gene sequences, and, as shown in FIG. 18, RNA from both stimulated and unstimulated TH1 cells contained 200 gene mRNA, while none of the samples obtained from TH2 cells contained 200 gene mRNA. It should also be noted that 200 gene expression
30 was higher in each of the stimulated TH1 cells relative to the corresponding unstimulated TH1 cells.

As shown in Table 1, above, the 54 gene is expressed in a TH1-restricted manner. The 54 gene was identified via T cell paradigm searches in which the expression pattern of a
35 TH1 cell clone, AE7, was compared to that of a TH2 cell clone, D10.G4. The initial differential expression analysis

was performed using differential display techniques, as
described, above, in Section 6.1.

The TH1-restricted pattern of the 54 gene expression was
corroborated through Northern analysis of RNA isolated from
5 TH1 cell lines (AE7, D1.1, Dorris) and TH2 cell lines
(D10.G4, DAX, CDC25), as shown in FIG. 19. The TH1/TH2
Northern blot data depicted in FIG. 19 additionally
illustrates 54 gene expression within cell clones either
stimulated or unstimulated with anti-CD3 antibodies, and
10 demonstrates that 54 gene expression goes down within
stimulated TH1 cells.

To further characterize the 54 gene expression, a
detailed time course study was conducted using RNA isolated
from AE7 clones. Specifically, RNA was isolated from
15 unstimulated AE7 clones as well as from AE7 clones which had
been stimulated with anti-CD3 antibodies for varying lengths
of time, as noted in FIG. 20. As illustrated in FIG. 20, 54
gene expression decreased slightly by 2-6 hours after
stimulation and had not again achieved pre-stimulation levels
20 within 48 hours after stimulation.

A 54 gene expression analysis of cell lines representing
a variety of T cells, B cells and monocytic/macrophage cell
lines was performed which failed to detect 54 gene expression
in non-TH1 cells, demonstrating that 54 gene expression is
25 highly restricted to TH1-like cells. A Northern analysis of
54 gene expression within tissues (FIG. 21), also
demonstrated an expression profile consistent with that of a
TH1 cell-restricted expression profile. Namely, as shown in
FIG. 21, most organs failed to express the 54 gene, while the
30 highest level of 54 gene expression was seen in lymph node
tissue, and lowest detectable level of expression was seen in
spleen, testis and uterus.

Band 54 nucleotide sequence, which had been obtained
from the amplified cDNA produced in the initial differential
35 display analysis in which the 54 gene was identified, was
used to isolate seven cDNA clones, designated 54A-G. Each of

the clones were of similar size. The 54-C cDNA has been
deposited with the NRRL within the E. coli clone, 54-C.

FIG. 22 shows the entire 54 gene coding sequence (SEQ ID
NO:11). The derived amino acid sequence of the 54 gene
5 product is also shown in FIG. 22 (SEQ ID NO:12). Based on
database homology searches, the 54 gene appears to encode a
novel cysteine protease. Cysteine proteases are enzymes
which contribute to intracellular protein degradation and
appear to play a role in tissue degradation. It is possible,
10 therefore, that the inhibition of 54 gene expression and/or
54 gene product activity in immune disorders involving TH1-
like cells may serve to minimize any tissue damage.

Specifically, the 54 gene sequence exhibits the three
thiol protease domains typical of active cysteine protease
15 enzymes. These domains include a CYS daomain at
approximately amino acid residue 145 to 156 (active site: C,
position 151), a HIS domain at approximately amino acid
residue 287 to 297 (active site: H, position 289), and an ASN
domain at approximately amino acid residue 321 to 340 (active
20 site N, position 326). Interestingly, the typical CYS domain
is broken by a K residue at position 149 (this position is
usually G or E), perhaps indicating that the 54 gene product
cysteine protease is very substrate-specific. Additionally,
amino acid sequence analysis indicates probable disulfide
25 bonds between cysteines at 148 and 189, 182 and 224 and 282
and 347. Further, FIG. 23 depicts the 54 gene product amino
acid sequence and points out some of its potential cysteine
protease-like features. For example, the 54 gene product has
an amino terminal end which resembles a cysteine protease
30 preproenzyme region, which is cleaved away upon formation of
the active cysteine protease. The boxed region, from amino
acid residue 56 to 75 represents an "ERFNIN" region which has
previously been noted as a feature of several cysteine
proteases (Ishidoh, K. et al., 1987) FEBS Lett. 226:33-37).
35 The circled amino acid residues within the boxed region
represent conserved amino acid residues. The individual
boxed amino acid residues represent residues that, based on

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homology, are thought to lie within the active site of the
enzyme.

9.   EXAMPLE: IDENTIFICATION AND CHARACTERIZATION OF HUMAN
                            200 GENE

5   In the Example presented herein, the cloning,
identification and characterization of the human 200 gene,
corresponding to the human homolog of the murine 200 gene, is
described.

10
                    9.1. MATERIALS AND METHODS
          Murine 200 gene probe: An approximately 800 bp EcoRI
insert containing about 90% of the murine 200 gene cDNA
(femt200) ORF was gel purified, ^{32}P labelled, and used to
15 probe the λgt11 human lymphocyte cDNA library described
below.
          Human 200 gene probe:
          The approximately 500 bp insert of the human 200 gene
feht200a cDNA clone was ^{32}P labelled and used to probe the
20 human fetal spleen cDNA library described below.
          Screening procedures: Approximately 10^6 plaques of a
λgt11 human lymphocyte cDNA library (Catalog No. HL 1031B;
Clontech) were screened with murine 200 gene probe described
above in duplicate. The filters were hybridized with probe
25 overnight at 65°C in Church's buffer (7% SDS, 250mM NaHPO$_4$,
2μM EDTA, 1%BSA). The next day, filters were washed in
2XSSC/1% SDS for 30 min at 50°C. The filters were then
exposed to Kodak film at -80°C. Positive plaques were
rescreened under the same conditions.
30   A human fetal spleen cDNA library constructed using the
Stratagene Uni-Zap cloning System was screened using the
human feht200a gene probe described above. Approximately 10^6
plaques were hybridized in duplicate at 65°C in Church's
buffer overnight. The filters were then washed for 30 min at
35 65°C in 0.1XSSC,0.1% SDS and exposed to film. Positives were
confirmed by secondary screening under the same conditions.

Subcloning/Sequencing procedures:

DNA from the positive clones obtained from the λgt11 cDNA library was generated by a plate lysis method. The purified DNA was digested to obtain cDNA inserts which were
5 subcloned into the pBluescript plasmid (Stratagene).

Positive clones obtained from the human fetal spleen cDNA library were excised with ExAssist helper phage, XL1-Blue cells and SOLR cells as described by Stratagene. Excision products were then plated out on LB/Amp plates and
10 incubated at 37°C overnight. White colonies were picked and DNA prepared for sequencing.

DNA sequencing was performed according to standard techniques.

Northern blot analysis of human gene 200 expression:
15 Northern blots were carried out as described in Section 6.1, above. 15 μg of total RNA from a variety of human organs were analyzed (Clontech, CA). The ^{32}P labelled probe utilized was the feht200a clone, described above, which contains the 5' ORF of human gene 200.
20

9.2 RESULTS

The full length sequence of the human 200 gene was successfully cloned and characterized, as described herein.

In order to clone human 200 gene, an 800 bp EcoRI insert
25 containing approximately 90% of the murine 200 gene cDNA (femt200) ORF was gel purified, ^{32}P labelled, and used to probe a λgt11 human lymphocyte cDNA library. Approximately 10^6 plaques were screened in duplicate, as described in Section 9.1, above. One positive plaque was obtained and
30 rescreened under the same conditions. Once pure, this clone was used to generate lambda DNA by a plate lysis method, and the lambda DNA was digested to obtain a 500 bp insert (feht 200a) which, upon sequencing, was found to be a human homologue of the murine 200 gene.
35   To obtain a clone encoding the entire ORF of the human 200 gene, a human 200 gene probe was used to screen a human fetal spleen cDNA library, as described in Section 9.1.,

above. Three positive clones were obtained, two of which
were positive upon secondary screening under the same
conditions. The two positive clones were subcloned and their
cDNA inserts were sequenced. These two clones labelled
5 feht200b and feht200c were approximately 1.56 kb and 2.0 kb
in length, respectively with feht200c containing the entire
coding sequence. Clone feht200c was deposited with the ATCC,
as described, below, in Section 12.

The nucleotide sequence containing the complete human
10 200 gene open reading frame is depicted in FIG. 24 (SEQ ID
NO: 37). The derived amino acid sequence of the human 200
gene product is also depicted in FIG. 24 (SEQ ID NO: 24).

The 301 amino acid residue sequence of the human 200
gene product reveals that it is a cell surface receptor
15 exhibiting distinct domains, including a signal sequence from
amino acid residue 1 to approximately 20, an extracellular
domain from approximately amino acid residue 21 to 200, a
transmembrane domain from approximately amino acid residue
201-224 and a cytoplasmic domain from approximately amino
20 acid 225 to 301. The extracellular domain contains an Ig
type variable set domain from approximately amino acid
residue 30 to approximately amino acid residue 128, thus
placing the 200 gene product within the Ig receptor
superfamily.

25   A Northern analysis of the tissue distribution of 200
gene transcripts was performed. 15 μg RNA from brain,
kidney, liver, lung, muscle, prostate, spleen, thymus and
trachea were isolated and analyzed for human 200 gene
expression. This analysis revealed human 200 gene
30 transcripts of approximately 2.2 kb, in tissues including
brain, lung, trachea, spleen and thymus.

In summary, the human 200 gene, corresponding to the
human analog of the murine 200 gene, has been successfully
cloned and characterized, as described herein. As revealed
35 by its amino acid sequence, the human 200 gene product is a
receptor of the Ig superfamily class of molecules.

10. <u>EXAMPLE: CONSTRUCTION AND EXPRESSION OF IgG1 FUSION</u>
<u>PROTEINS</u>

Described in this Example is the construction and
expression of IgG1 fusion proteins. Specifically, the
5 construction of human and murine 200 gene and 103 gene IgG1
fusion proteins are discussed.

10.1. <u>MATERIALS AND METHODS</u>
<u>Recombinant plasmids encoding IgG1 fusion proteins</u>:
10   <u>Generation of the vector encloding murine 200 gene-hIgG1</u>
<u>fusion protein</u>: The fragment encoding the signal sequence
and extracellular domain of murine 200 gene was amplified
from a cDNA clone containing the ORF of murine 200 gene using
the following oligonucleotides:

15
<u>Forward oligo</u>: 5'-AAA-TTT-ATT-CTC-GAG-GAC-CCA-CGC-GTC-CGG-
ATT-TCC-C-3' (SEQ ID NO: 25);
<u>Reverse oligo</u>: 5'-TTA-ATT-TGG-ATC-CCC-AGT-TCT-GAT-CGT-TTC-
TCC-AGA-GTC-3'(SEQ ID NO: 26).

20
The oligonucleotide primers also introduce XhoI and BamHI
restriction sites at the 5' and 3' ends of the PCR products,
respectively, to facilitate the subsequent insertion into
IgG1 expression vectors (pCD5-CD44-IgG1; see Aruffo, A. et
25 al., 1991, Cell <u>61</u>:1303-1313). The pCD5-CD44-IgG1 vector
encodes a protein containing a CD5 signal sequence, a CD44
extracellular domain and a human IgG1 heavy chain Fc region.
For construction of the murine 200 gene-hIgG1 fusion protein
vector, the CD5 and CD44 portions of pCD5-CD44-IgG1 were
30 replaced with sequences encoding murine 200 gene product
signal sequence and extracellular domain.
' The PCR reactions consisted of 25 cycles amplification
at an annealing temperature of 60°C. Vent™ thermostable DNA
polymerase (New England BioLabs, Inc.; Beverly,
35 Massachusetts) was used in the amplification. The PCR
product (approximately 600 bp) was digested with XhoI and
BamHI and inserted into pCD5-CD44-IgG1 previously digested

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with XhoI and BamHI to remove the sequences encoding the CD5
signal sequence and the CD44 ectodomain.

    <u>Generation of the vector encoding human 200 gene-hIgG1
fusion protein</u>: The fragment encoding the signal sequence
5 and extracellular domain of human 200 gene is amplified from
a cDNA clone containing the ORF of human 200 gene using the
following oligonucleotides:

<u>Forward oligo</u>: 5'-AAA-TTT-ATT-CTC-GAG-CGC-TAA-CAG-AGG-TGT-CC-
10 3' (SEQ ID NO: 27);
<u>Reverse oligo</u>: 5'-TTA-ATT-TGG-ATC-CCC-TCT-GAT-GGT-TGC-TCC-
AGA-GTC-CCG-3' (SEQ ID NO: 28).

The amplification and pCD5-CD44-IgG1 subcloning procedures
15 are as described, above, for the murine 200 gene-hIgG1 fusion
protein.

    <u>Generation of the vector encoding the murine 103 gene-
hIg G1 fusion protein</u>: The construction of a vector encoding
a soluble Ig-fusion protein (size: approximately 60 kD)
20 containing a murine 103 gene product extracellular domain
(but lacking the 103 gene product signal sequence) was
constructed as described here. The CD44 portion of the pCD5-
CD44-IgG1 vector (described above) was replaced with a
nucleotide sequence encoding the 103 gene product
25 extracellular domain. The 103 gene product extracellular
domain sequence of the Ig-fusion protein consisted of 103
gene product amino acid residues 27-342 (i.e., the 103 gene
product portion ending with amino acid sequence Ile-Val-Ala-
Gly-Cys-Ser).

30   The fragment encoding the 103 gene product extracellular
domain was amplified by PCR using synthetic oligonucleotides
complementary to the sequences flanking the 103 gene region
that would produce the 103 gene product containing amino acid
residues 27-342. The oligonucleotides were designed to allow
35 creation of a KpnI site at the 5' end and a BamHI site at the
3' end of each amplified 103 gene fragment to facilitate
subsequent insertion into pCD5-CD44-IgG1.

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The 5' oligonucleotide was as follows:
5'-CCGCGGGTACCAGTAAATCGTCCTGGGGTGG-3' (SEQ ID NO: 29).
The 3'oligonucleotide was as follows: 5'-
AAATAAAGGATCCCTACATCCAGCAACTATGTAGTA-3' (SEQ ID NO: 30).

5   PCR reaction conditions consisted of 15 cycles of 30
seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C,
using Vent DNA polymerase (New England Biolabs, Beverly, MA)
and 103L gene as template.

103 PCR products were digested with KpnI and BamHI, and
10 ligated to KpnI-BamHI sites of CD5-IgG1 vector, thus
replacing the CD44 sequences with the 103 gene sequences.

The resulting plasmid, encoding a fusion protein
containing CD5-signal sequence, murine 103-extracellular
domain and human-IgG1 heavy chain Fc region, was transfected
15 into COS cells using LipofectAMINE™ (GIBCOBRL, MD) following
manufacturer's suggest. 0.18μg plasmid DNA and 140μl
LipofectAMINE™ were used for transfection of the cells of a
150mm plate. Twenty-four hours after transfection, medium
was replaced with 10% Ultra-low IgG Fetal Bovine Serum
20 (GIBCOBRL, MD)/DMEM(BioWHITTAKER, Maryland), and the
transfected cells were allowed to grow for 4-5 days
continuously. Supernatants were then harvested, centrifuged
to remove nonadherent cells and debris, and stored at -20°C.

For purification, 1ml of supernatant was precipited
25 overnight with 10μl of IPA-300 Immubilized rProteinA
(Repligen, MA) at 4°C. The next day, beads were collected by
centrifugation and washed three times with 10 volumes of PBS.
For analysis, the beads were suspended in 20μl of 2 X Laemmli
Sample Buffer (BIO-RAD, CA) and boiled at 100°C for 10 min.
30 The boiled sample was spun briefly and loaded onto a 10% SDS-
PAGE gel (JILEinc. CT).

Metabolic labelling of recombinant fusion proteins: 36
hours after transient transfection of COS-7 cultures, cells
were rinsed with replacement growth medium [DMEM methionine
35 and cysteine depleted (ICN, Inc., CA)]. After rinsing, 150
μCI/ml medium of a mixture of ^{35}S-cysteine and ^{35}S-methionine

(Express $^{35}S^{35}S^{TM}$, Dupont, MA) was added to the replacement medium and the cells were cultured overnight.

Analysis of recombinant proteins by SDS PAGE:

hIgG1 fusion proteins were generated by LipfectAMINE™
5 (Gibco, Inc., MD)-mediated transient transfection of COS-7 cells according to manufacturer's suggestion for 200 gene-hIgG1 fusion proteins, 1 ml of day 5 supernatant was mixed with 20μl of Protein A Trisacryl bead (Pierce, Inc., IL) in the presence of 20mM HEPES (pH 7.0) overnight at 4°C with
10 constant agitation. Beads were then washed 3X with PBS prior to the addition to loading buffer. Beads were mixed with either reducing or non-reducing loading buffers (described in, Molecular Cloning, Sambrook, Fritsch, and Maniatis, 2nd edition, 1989, with the exception that DTT was replaced with
15 2.5% β-mercaptoethanol).

10.2. RESULTS

The construction and expression of recombinant IgG fusion proteins is described herein. Specifically, 200 gene
20 product-IgG1 and 103 gene product-IgG1 fusion proteins are described. The murine and human 200 gene product-IgG1 fusion protein contains a 200 gene product signal sequence and extracellular domain fusion to a human IgG1 heavy chain Fc region. The 103 gene product-IgG1 fusion protein contains a
25 CD5 signal sequence and 103 gene product extracellular domain fused to a human IgG1 heavy chain Fc region.

200 gene-hIgG1 fusion proteins were produced by transient transfection of COS-7 cells, as described in Section 10.1, above. Protein A immunoprecipitation of the
30 COS-7 supernatants and their analysis by SDS-PAGE demonstrated, first, that the corect IgG-1 peptide was being produced as part of the fusion (as evidenced by the fusion's protein A immunoprecipitation) and, second, demonstrated substantial expression of the 200 gene-IgG1 fusion protein at
35 a concentration approximately 1μg per ml of culture supernatant. Further, when the immunoprecipitated supernatants are analyzed and compared under reducing and

non-reducing conditions, it is clear that the 200 gene-IgG1
fusion protein undergoes oligomerization, as expected, given
the human IgG1 heavy chain peptide sequence present in the
fusion protein. Further, the size (*i.e.*, larger than

5 expected from the amino acid sequence alone) and appearance
of the fusion proteins as they migrate through the gels
(*i.e.*, diffuse, rather than tight bands) indicate that, as
expected, the fusion proteins have been glycosylated.

10 11. EXAMPLE: PRODUCTION AND CHARACTERIZATION OF TRANSGENIC
ANIMALS
        Described herein is the production and characterization
of transgenic mice overexpressing either murine 200 gene
product or murine 103 gene product.

15
11.1. MATERIALS AND METHODS
Construction of 200 gene transgenic clone:
        A PCR product of the entire 200 gene sequence was used
to replace the IL-10 gene in the pCIL-10 plasmid, whose
20 construction is described below.
        The pCIL-10 plasmid contained a 5.5 kb *Bam*HI-*Xba*I
genomic fragment, within which human CD2 enhancer was
included (Greaves et al., 1989, Cell 56(6):979-86). A 0.5 kb
*Xba*I-*Sma*I fragment containing human immunoglobulin heavy
25 chain promoter, Pμ (Danner and Leder, Proc. Natl. Acad. Sci.
USA, 1985, 82:8658-8662), was ligated to the 3'-end of the
CD2 fragment. Following the Pμ fragment was a *Xba*I (blunt-
ended)-*Bam*HI fragment containing the IL-10 coding sequence,
to which was ligated the 2.1 kb *Bam*HI-*Eco*RI genomic fragment
30 of human growth hormone (Base 5164 to 7317 of HUMGHCSA
(GenBank)) at the 3'-end of the construct.
        A 0.8 kb PCR product of the entire murine 200 gene
coding sequence was obtained through 25 cycle-reaction using
the murine 200 gene cDNA 200-AF as template and
35 oligonucleotides primers with compatible restriction sites
*Spe*I at the 5'-end and *Bam*HI at 3'-end. The 5'-oligo
utilized was 5'-GCG CAA TTG ACT AGT GAC CCA CGC GTC CGG ATT

TC-3' (SEQ ID NO: 31) and the 3'-oligo, 5'-GAC GCG GAT CCT
CAG GAT GGC TGC TGG CTG-3' (SEQ ID NO: 32). After heat
denaturation at 95°C for 2 minutes, 3-step cycling was
performed for 30 seconds at 95°C, 30 seconds at 60°C, 60
5 seconds at 72°C by Vent™ DNA polymerase (New England Biolabs,
MA). A final step for five minutes, at 72°C, was performed
for end-polishing. The PCR product was digested by SpeI and
BamHI (New England Biolabs, Beverly, MA) and ligated to the
fragment of pCIL-10 after removal of SpeI to BamHI of IL-10
10 gene. MaxEfficient E. coli DH5α competent cells (GIBCO BRL,
MD) were used for transformation following manufacturer's
suggestion. Transformants were grown in LB broth containing
0.1 μg/ml ampicillin and the DNA were extracted by Qiagene
Plasmid Maxi Kit (Qiagene, CA). Restriction analysis was
15 performed for confirmation, and the final construct was
sequenced to eliminate any possible PCR introduced mutations.
A plasmid designated p200Tr3 was selected from production of
transgenic mice.

This final construct contained an approximately 5.5 kb
20 genomic fragment containing the human CD2 enhancer joined to
a 0.5 kb fragment of the human IgM promoter immediately
upstream of the murine 200 gene coding sequence. A region
containing the 3' untranslated sequence of the human growth
hormone gene was positioned immediately downstream of the
25 murine 200 gene ORF and contained a polyA splice site.

Construction of 103 gene transgenic clone:

A PCR product of the entire 103 gene sequence was used
to replace the IL-10 gene in the pCIL-10 plasmid. The
pCIL-10 plasmid was as described in this Section, above. A
30 PCR product of the entire murine long form of the 103 gene
(Yanagisawa, K. et al., 1993, FEBS 318:83-87) coding sequence
was obtained through 35 cycle-reaction using first-strand
cDNA from a mouse TH2-type cell line, D10G4 (ATCC, MD), as
template. Total RNA was extracted from the cell line by
35 RNAzole™ (TEL-TEST, Inc., TX). Seven micrograms RNA were
used in a 20 μl first-strand cDNA synthesis reaction by
Superscript Reverse Transcriptase I (GIBCO BRL, MD) following

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manufacturer's suggestion. Two microlitters of cDNA were
used in PCR reaction. The 5'-oligo was
5'-GAACACACTAGTACTATCCTGTGCCATTGCCATAGAGA-3'(SEQ ID NO: 33),
and the 3'-oligo,

5   5'-GGAATATTGGGCCCTTGGATCCCAAGTCTGCACACCTGCACTCC-3' (SEQ ID
NO: 34) with compatible restriction sites *SpeI* at 5'-end and
BamHI at 3' end, respectively. After heat denaturation at
95°C for 2 minutes, 3-step cycling was performed at 45
seconds at 95°C, 45 seconds at 65°C and 60 seconds at 72°C by

10 Vent™ DNA polymerase (New England Biolabs, Beverly, MA). A
final step for five minutes, at 72°C, was performed for end-
polishing. The PCR product was digested by *SpeI* and *BamHI*
(New England Biolabs) and ligated into the *SpeI-BamHI* sites
of pBSKIIGH vector, containing the human growth hormone

15 fragment from pCIL-10 subcloned into the *BamHI-XhoI* site of
pBSKII (Stratagene), which was named pBS-103L-GH. The
pCIL-10 fragment containing human CD2 enhancer and Pμ
promoter was then ligated immediately upstream of the 103L
gene of pBS-103L-GH. MaxEfficient *E. coli* DH5α competent

20 cells (GIBCO BRL, MD) were used for transformation following
manufacturer's suggestion. The transformants were grown in
LB broth containing 0.1 μg/ml ampicillin and DNA were
extracted by Qiagene Plasmid Maxi Kit (Qiagene, CA).
Restriction analysis was performed for confirmation, and the

25 construct was sequenced to eliminate any possible PCR
introduced mutations. A plasmid designated pCD2-103L-GH was
selected for production of transgenic mice.
<u>Production of Transgenic Mice</u>

        C3H/HEJ and FVB/NJ mice were obtained from the Jackson

30 Laboratory (Bar Harbor, ME). Females aged 3-4 weeks were
induced to ovulate by intraperitoneal injection of pregnant
mare's serum (PMS) between 10 a.m. to 2 p.m., followed 46
hours later by intraperitoneal injection of human chorionic
gonadotropin (hCG). Following hCG administration, the

35 femalec were housed overnight with males of the same strain.
The following morning females were examined for the presence
of a copulation plug and embryos were isolated from those

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females with plugs, essentially as described in *Manipulating
the Mouse Embryo* (Hogan et al., eds., Cold Spring Harbor
Laboratory Press, 1994).

 DNA for embryo microinjection was prepared by digesting
5 of p200Tr3 and pCD2-103L-GH1 with *Not*I and *Xho*I followed by
gel electrophoresis. The 9 kb and 10 kb fragments,
respectively, were electrophorese onto an NA-45 membrane
(Schleicher and Schuell) by cutting a slit into the gel
immediately in front of the desired band, inserting the NA-45
10 membrane and continuing electrophoresis until the DNA band
has been transferred to the membrane. The DNA was eluted
from the membrane by incubation with 0.4 ml of 1M NaCl/0.05M
arginine-free base at 65-70°C for several hours in a
microfuge tube. The eluted DNA was extracted with
15 phenol/chloroform and chloroform, ethanol precipitated and
dissolved in 200 μl of 5 mM Tris, pH 7.5/0.1 mM EDTA. The
DNA was then re-precipitated with ethanol and re-dissolved in
40 μl of 10 mM Tris, pH 7.5/0.1 mM EDTA. Prior to
microinjection, the DNA was diluted to 1-2 μ/ml in 10 mM
20 Tris, pH 7.5/0.1 mM EDTA.

 DNA was microinjected into the male pronuclei of strain
C3H/HEJ or FVB/NJ embryos and injected embryos were
transferred into the oviducts of pseudopregnant females
essentially as described in *Manipulating the Mouse Embryo.*
25 The resulting offspring were analyzed for the presence of
transgene sequences by Southern blot hybridization of DNA
prepared from tail biopsies.
Southern blot analysis of transgenic mice:
 Approximately 1/2" piece of tail was clipped and
30 digested in 500 μl proteinase K solution [containing 100 mM
Tris HCl, pH 8.0; 5 mM EDTA, pH 8.0; 0.2% SDS; 200 mM NaCl;
100 μg/ml Proteinase K (Boehringer Mannheim, Germany)] at
55°C overnight. Digests were centrifuged for 15 minutes to
remove undigested debris. Supernatants were precipitated
35 with an equal volume of isopropanol at room temperature.
Precipitates were centrifuged for 25 minutes and pellets
washed in 75% ethanol. Pellets were air dried and

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resuspended in 100 μl TE; pH 8.0. Restriction digests of
tail DNA were performed as follows: 20 μl DNA solution was
digested with 80 units BamHI (New England Biolabs) in the
presence of 1 mM spermidine overnight at 37°C. Digested
5 samples were analyzed by gel electrophoresis using 0.8%
agarose gels. Separated DNA was transferred to Hybond-N+
(Amersham, Inc.) following depurination in 0.25M HCl for 10
minutes followed by 0.5 M NaOH, 1 M NaCl for 30 minutes, and
then 2.5M Tris-HCl (pH 7.4), 2.5M NaCl for 30 minutes.
10 Immediately prior to transfer, gels were briefly equilibrated
in a 10X SSC transfer buffer. Transfer was carried out
overnight in 10X SSC by capillary action. After transfer,
the membrane was air dried and UV-crosslinked using a
Stratolinker (Stratagene, Inc.). After crosslinking,
15 membranes were rinsed briefly in 2X SSC.

For 200 gene transgenic analysis, radiolabelled probe
containing approximately 500 base pairs of the human IgM
promoter was produced using the Random Primed DNA Labelling
Kit (Boehringer Mannheim). The 500 bp *Xba*-1/*Spe*-1 fragment
20 of human IgM heavy chain promoter was used as probe.
Hybridization was carried out using standard hybridization
procedures using Rapid-Hyb (Amersham) hybridization solution.
1×10^6 cpm per ml of hybridization solution was incubated at
65°C overnight. Membranes were washed twice in 0.5X SSC 0.1%
25 SDS at 65°C for 30 minutes and were exposed by
autoradiography. Transgenic animals were detected by the
presence of an approximately 7.0 kb BamHI fragment which
hybridizes to a probe containing the 0.5 kb Pμ fragment.

For 103 gene transgenic animals, a ^{32}P-radiolabelled PCR
30 fragment of the pCD2-103L-GH construct described above was
utilized. The PCR fragment was generated using the following
primers: 5' oligo: 5'-GTA-AAT-CGT-CCT-GGG-GTC-TGG-3'(SEQ ID
NO:35; 3' oligo: 5'-CCT-TCT-GAT-AAC-ACA-AGC-ATA-AAT-C-3'
(SEQ ID NO:36). Using these oligonucleotide primers and the
35 pCD2-103L-GH template, PCR reactions conditions were as
follows: 20 cycles of 30 seconds at 94°C, 30 seconds at 60°C
and 30 seconds at 72°, using Vent™ DNA polymerase (New

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England Biolabs, Beverly MA). Upon hybridization to mouse
genomic digested with EcoRI and SpeI, the resulting probe
hybridized to an endogenous 2.4 kb band and a 0.85 kb
transgenic-specific band.

5

11.2. RESULTS

200 gene transgenic mice (four C3H founder lines, 6 FVB
founder lines) and 103 gene transgenic mice (five FVB
founder lines) were produced according to the method
10 described above, in Section 11.1. Southern hybridization
analysis demonstrated the successful production of both 200
and 103 gene transgenic founder animals.

With respect to the 200 transgenic animals, four lines
of transgenic mice were created in the C3H inbred strain of
15 mice. One of these lines was examined for expression of the
200 transgene. As expected, 200 transgene transcripts were
detected in the thymus, spleen and lymph nodes, consistent
with a predominantly T-cell restricted pattern. At
approximately 6 to 7 months of age, three of the founder
20 animals, upon visual examination, appeared sick. One of
these founders, designated 130-1.2, was sacrificed at
approximately 6 months of age. At the time the sacrifice, it
was expected that at the female would not have lived
significantly longer. Upon dissection of 130-1.2, it was
25 clear that the spleen and one of the kidneys were grossly
abnormal. The spleen was approximately ten-fold normal size
and appeared to be filled with pale appearing cells. The
splenocyte populations were examined by flow cytometry, and
it was determined that the predominant cell population was
30 positive for MAC-1 (a macrophage/granulocyte cell surface
marker) expression. These cells also had high side scatter
profiles. Spleen sections from this animal were stained with
hematoxylin and eosin and viewed by light microscopy. These
data suggest that the abnormal cell population was composed
35 of polymorphonuclear neutrophils. The abnormal kidney also
appeared to be infiltrated by these same cells.

One of the offspring of 130-1.2 died at approximately
6 months of age while giving birth to her second litter.
Upon dissection, it was noted that there appeared to be a
bowel obstruction, which may have contributed to the cause of

5 death. In addition, yet another founder animal appeared to
be quite sick and was sacrificed. However, in this animal
there were no abnormalities observed, either by gross
inspection of the organs or by flow cytometric analysis of
lymphoid populations. Finally, the remaining founder animal

10 was observed to be exhibiting symptoms of sickness by
approximately 6 months of age.

Given that these animals were maintained under SPF
(specific pathogen free) conditions, it is highly unlikely
that these animals became ill via exposure to an infectious

15 pathogen. Rather, it is most likely that the effect of the
transgene is modulating some aspect of the immune system.
Based on the observation of 130-1.2, it is suspected that as
a consequence of transgene expression, the line may suffer
from an immunodeficiency and is, therefore, susceptible to

20 infection by normally innocuous organisms present in the
environment (bacteria, etc.). It is possible, therefore,
that this gene product normally functions in some aspect of
the immune effector response or in the proper regulation of
the immune system.

25 Two hundred transgenic mouse founder lines generated in
the FvB inbred strain exhibited no outward symptoms of
illness as they approached 6 months of age.

12. EXAMPLE: THE 103 GENE PRODUCT EXHIBITS A CRITICAL ROLE
     IN REGULATING TH2 EFFECTOR CELL RESPONSES

30 The Example herein presents in vivo data demonstrating
that the 103 gene product regulates TH2 effector cell
responses. In particular, a monoclonal antibody (3E10 mAb)
has been generated against the 103 gene product, and its

35 effect in an adoptive transfer model of TH1 and TH2 immune
responses was investigated. The effect of a 103 gene product

fused to an Ig tail (103/Ig fusion) has also been studied in the adoptive transfer model.

The anti 103 gene product mAb abrogated the production of IL-4, IL-5, IL-6 and IL-13, TH2 mediated lung inflammation
5 and the associated airway hyperresponsiveness. Likewise, the 103/Ig fusion results in a decrease in eosinophil infiltration into and inflammation of lung airways. In contrast, the 103 gene product mAb failed to inhibit TH1-mediated lung pathology and IFN-γ secretion.
10 These results, therefore, provide in vivo animal data indicating that the 103 gene product provides a critical signal to TH2 effector cells and can be utilized as a novel target for the selective suppression of TH2 immune responses.

15   12.1.   <u>MATERIALS AND METHODS</u>

<u>CD3/TCR Crosslinking</u>: Mice expressing the transgene for the DO11.10 αβ-TCR, which recognizes residues 323-339 of chicken ovalbumin (OVA) in association with I-A^d (Murphy, K.M., et al., 1990, Science <u>250</u>:1720-1723) were utilized.
20 DO11.10 TCR-transgenic CD4$^+$ T cells were cultured in complete RPMI 1640 with OVA$_{323-339}$ (1 μM) and mitomycin C-treated splenocytes. For TH1 phenotype development, recombinant murine Il-12 (10ng/ml) and neutralizing anti-IL-4 mAb (11B11, 40μg/ml, R&D Systems) were added and for TH2 development
25 recombinant murine IL-4 (10ng/ml) and neutralizing polyclonal anti-murine IL-12 (TOSH-2, 3μg/ml, Endogen, Cambridge, MA) were used. Cultures were maintained for 48 hours and 5 days after stimulation, after which time cells were harvested and purified over ficoll. 1 x 10^7 cells were washed and RNA
30 extracted as described below. The remainder of the cells were stimulated on plate bound anti-CD3 in the presence of h IL-2 (Endogen) for 48 hrs.

<u>anti-103 Ab</u>: Rat monoclonal antibodies (MAbs),
35 including the 3E10 MAb, were generated against the extracellular domain of the mouse 103 gene product. A DNA sequence containing the extracellular domain of 103 gene

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product was PCR-amplified and cloned into a vector containing the CD5 signal sequence and the human IgG1 constant region. COS cells were transiently transfected using lipofectamine™ (GIBCO) protocol according to manufacturer's instructions.

5 Cells were cultured in Ultra-Low™ Ig fetal bovine serum (GIBCO) for approximately one week prior to harvest.and the recombinant protein was purified by passage over a protein A column.

Lou/M rats were then immunized by subcutaneous injection 10 of 0.5 mg purified recombinant 103 gene product protein. Rats were boosted twice via intraperitoneal injections at 2 week intervals with approximately 300 μg purified protein. Animals were analyzed for reactivity to the fusion protein by FACS and ELISA approximately 10 days after the last boost.

15 Four weeks later, positive reacting animals were boosted once more and sacrificed 3 days later. Splenocytes were fused with SP/2 myeloma cells and resulting clones were screened and selected to be specific for the 103 gene product on the basis of their reactivity against 103 gene product Ig, but not 20 CD44-Ig, and their ability to stain 103 gene product COS transfectants, but not control transfectants. Pre-immune serum from non-immunized Lou/M rats was used as negative controls.

One of these mAbs was identified and termed 3E10.

25

Surface Expression Of 103 Gene Product On
TH2 Clones And TH2 Effector Cells

The 3E10mAb was labeled with digoxigenin and the number of 103-positive cells were detected by anti-digoxigenin Fab 30 fragments (Boehringer Mannheim) conjugated to Cy5.

CD4 positive, L-selectin negative cells were isolated using high gradient magnetic cell separation system MACS (Milteny; Biotec, Berg-Gladbach). Expression of 103 was analyzed on a fluorescence-activated cell sorter (FACS)- 35 calibur (Becton-Dickinson) five to seven days after restimulation with OVA peptide under the indicated polarizing conditions.

In Vitro Activation Of TH2 Effector Cells

TH2 effector cells were activated with platebound CD3
(1µg/ml, 2C11) and CD28 (37.51, 4µg/ml), Pharmingen, San
Diego) and 3E110 (20 µg/ml) for 48 hours. IL-4 and IL-5
5 levels were measured in the supernatent by Elisa.

RNA isolation: Total cellular RNA for RT-PCR analysis
was extracted from cells using the Rneasy Total RNA kit
(Qiagen; Chatsworth, CA). Poly A+ RNA (for Northern
10 analysis) was isolated from activated cells using FastTrack
mRNA Kit (Invitrogen Corp.; San Diego, CA).

Northern Analysis: 1.0 µg RNA were loaded per lane for
the Northern blot analysis. The 103 gene probe was a 409 bp
15 RsaI fragment from the 103 gene cDNA (position 1252-1661
based on the published sequence for Genbank accession number
D13695). IL-4 and beta-actin probes were purchased from
Clontech, Inc., Palo Alto, CA. IFN-γ probe consisted of a
344bp fragment of murine IFN-γ covering the region from
20 position 532-876 (genbank accession number M28621). Northern
blot analysis was carried out according to standard
techniques.

RT-PCR: First strand cDNA was synthesized from equal
25 amounts of RNA using the Superscript Preamplification System
(Life Technologies; Gaithersburg MD). PCR was performed
using 25 ng of first-strand cDNA. The following gene-
specific primers were used for PCR amplification: Gene 103:
5' ACGGAGGGCAGTAAATC and 5' CAGCCAAGAAGTGAGAGC; IFN-gamma 5'
30 TGTTGCCGGAATCCAGCCTCAG and 5' GTCCCCCACCCCCAGATACAACC.
Primers for glyceraldehyde 3- Phosphate Dehydrogenase (G3PDH)
and IL-4 were purchased from Clontech Laboratories (Palo
Alto, CA). PCR was carried out using the Advantage KlenTaq
Polymerase mix (Clontech Laboratories; Palo Alto, CA)
35 according to the provided protocol; annealing temperature 56°
C. Samples were removed from the PCR reaction beginning
after 15 cycles and then after 5-cycle increments. Reactions

using the minimum number of cycles to visualize the gene of
interest, were loaded onto 1.5% agarose gels for analysis.

TH recipient mice: TH1 and TH2 subsets were generated
5 as described above. Mice were injected with 2 x 10⁶ TH2
cells intravenously into recipient BALB/c mice. Twenty four
hours later, mice were exposed daily to an aerosol of OVA (50
mg/ml) (Grade V, Sigma, St. Louis) for 20 min for 2
consecutive days. Control mice were either injected with TH2
10 cells and exposed to an aerosol of PBS or were exposed to OVA
in the absence of cell transfer. Mice were sacrificed 24 hrs
after the last aeroallergen challenge. One hr prior to
allergen exposure, mice were injected with either 20 µg or
100 µg of 3E10 mAb, recombinant 103 gene product-IgG fusion
15 protein, or 100 µg of rat IgG1 (Sigma, St. Louis) as the
appropriate isotype control. Twenty fours after the last
challenge, the trachea was cannulated and a bronchoalveolar
lavage performed with 4 x 0.3 ml aliquots of PBS (Gonazlo,
J.A., et al., 1996, Immunity 4:1-14). Cytokine levels in the
20 lavage fluid were measured by ELISA (PharMingen, San Diego).

Flow cytometry analysis of TH clones: AE7 (TH1), Dorris
(TH1), DAX (TH2) and D10.G4 (TH2) clones were analyzed for
the expression of gene 103 protein using fluorescence
25 activated cell sorting (FACS). Cells were stimulated with
appropriate antigen and cultured for approximately 3 days
prior to analysis. Pre-immune serum was prepared for
unimmunized Lou/M rats.
        50 µl of 3E10 culture supernatant (or 1 µg purified 3E10
30 protein) was applied 1X10⁶ cells. After rinsing, cells were
contacted with goat anti-rat antibody conjugated with PE (R-
phycoerythrin) fluorescent dye. After a final rinse, cell
analysis was carried out on a FACS Vantage (Becton
Dickenson).
35

103/Ig fusion protein: the 103/Ig fusion proteins were
generated as discussed, above, in the Example presented in
Section 10.

5   Animal Model Methods:
        Cell Preparation and polarization: Spleens from DO11.11
OVA αβ TCR mice were removed and CD4+ T cells were purified
by negative selection. Cells were plated at a density of 1 x
10^6/ml in 75 mm^2 flasks and stimulated with 10 μg/ml OVA
10 peptide and mitomycin C treated splenocytes at a ratio of 1:1
CD4: APC. Cells were cultured in the presence of IL-4 (20
ng/ml) and anti-IL-12 (3 μg/ml) for TH2 polarization, or IL-
12 (20 ng/ml) and anti-IL-4 (40 μg/ml) for TH1 polarization.
This procedure was repeated for 3 rounds of polarization.
15 Cells were then harvested, dead cells removed by density
centrifugation. TH1 and TH2 cells were then incubated at 1 x
10^6/ml for 48 hrs in IL-2 alone (10 ng/ml).

        Adoptive transfer model: 2 x 10^6 cells were injected
20 intravenously via the tail vein into recipient transgenic
mice. Twenty four hours later, mice were exposed daily to an
aerosol of OVA (50 mg/ml) antigen (Grade V, Sigma, St. Louis)
for 20 minutes. Control mice were exposed to an aerosol of
PBS alone. Mice were sacrificed on days 3, 5 and 7. In
25 separate experiments, mice received 20 μg/mouse i.v. of
either 3E10 MAb or the 103 Ig fusion protein. Control mice
were injected with 20 μg of either rat or human Ig as the
appropriate isotype control. This procedure was repeated for
two consecutive days.
30   24 hours after the last challenge, mice were
anaesthetized with 0.3 ml of 14% urethane i.p. and the
trachea cannulated. A bronchoalveolar lavage (BAL) was
performed by injecting 0.3 ml of PBS into the lungs. The
fluid was then withdrawn and stored on ice. This procedure
35 was repeated a total of 4 times. The cell suspension was
then centrifuged (5 mins, 1500 rpm, 4° C) and the supernatant
removed and frozen at -20° C. The cell pellet was then

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resuspended in 1 ml of PBS and total cell counts were
obtained. Cytospin preparations were then prepared and
stained with Diff-Quik (Baxter Corporation). A total of 200
cells were then counted differentially using standard
5 morphological criteria. Cytokine levels were measured in the
BAL fluid by ELISA (Pharmingen, San Diego).

     Active immunization protocol and IgE measurement: Male
BALB/c mice (15-20 g) were immunized intraperitoneally with
10 7.5 μg of OVA and 1.5 mg Al(OH)3 in saline on Day 0 and Day
7. On day 14 and Day 21 the mice were challenged with
aerosolized OVA (10 mg/ml) for 1 hours. Control mice were
challenged with PBS instead of OVA. One hour prior to each
allergen sensitization and challenge, the mice were injected
15 with 100 μg of 3E10 Mab or 100 μg of rat IgG1 (Sigma, St.
Louis). Twenty-four hours following the second allergen
challenge a BAL was performed and IL-5 levels in the BAL
fluid determined. Serum OVA specific IgE was determined by
specific ELISA.
20

     Airway responsiveness: Airway responsiveness was
measured in TH2 recipient mice, 24 hours after the last
aerosol challenge by recording respiratory pressure curves by
whole body plethysmography (Hamelmamn, J.E., 1997, Am. J.
25 Respir. Crit. Care Med. 156:766-775); Buxco®, EMKA
Technologies, Paris, France) in response to inhaled
methacholine (Aldrich-Chemie, Steinhein, Germany) at a
concentration of 2.5 to 25 mg/ml for 1 minute. This method
allowed measurements of spontaneous breathing in a non-
30 restrained mouse. Airway responsiveness was expressed in
enhanced pause (Penh), a calculated value, which correlates
with measurement of airway resistance, impedance and
intrapleural pressure in the same mouse. Penh = (Te/TR1) x
Pef/Pif (Te = expiration time, Tr = relaxation time, Pef =
35 peak expiratory flow, Pif = peak inspiratory flow)
(Hamelmamn, J.E., 1997, Am. J. Respir. Crit. Care Med.
156:766-775).

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Lung Histology: Following the BAL analysis, lungs were
inflated with 0.6 ml of a mixture of OCT compound (Tissue-
kek®; Miles Inc., Elkhart, IN) and 20% sucrose (Sigma, St.
Louis, MI) at a ratio of 1:1. The lungs were then removed,
5 snap-frozen and 8-10 μm cryosections fixed in methanol at
20°C for 2.5 minutes. Slides were stained with haematoxylin
and eosin (Fluka Chemika, Buchs, Switzerland).

In situ Hybridization: Recipient Balb/C mice were
10 injected intervenously with $2x10^6$ TH1 or TH2 cells generated
as described above. Twenty-four hours later, mice were
exposed to an aerosol of OVA (50 mg/ml; Grade V, Sigma) for
20 minutes for two consecutive days. Mice were sacrificed 24
hours after the last aeroallergen challenge. Lungs were
15 removed and snap frozen for in situ hybridization. A 35-mer
antisense oligonucleotide against 3'-UTR 103 gene sequence
was synthesized and end-labeled as follows: 100 pmol oligo
was incubated for 15 minutes at 37°C with 10 nmol dATP
(Promega), 40 μmol biotin-dUTP, 1x terminal transferase
20 buffer, 5 mM $CoCl_2$, 50 units transferase (Boehringer-
Mannheim, Germany). Formalin fixed 5 μm tissue sections were
hybridized for 16-18 hours. Control slides were hybridized
with probe mix containing 50-fold excess unlabelled oligo.
Hybridized probe was detected with a biotinyl tyramide
25 amplification method (GenPoint, Dako) and visualized by
adition of AEC substrate kit (Vector) for five minutes.

12.2. RESULTS

RT-PCR analysis performed herein demonstrates that the
30 103 gene is induced only upon CD3/TCR crosslinking during
differentiation of TH0 to TH2, but not TH1 effector cells.
The RT-PCR analysis was confirmed by Northern analysis.
These data corroborate the results presented in the Example
of Section 7, above.
35   To further investigate the expression and role of the
103 gene product in TH cells, a monoclonal antibody (3E10

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mAb) directed against the extracellular domain of the 103
gene product was prepared and characterized.

Flow cytometry data is presented in FIG. 25 which
demonstrates that the 3E10 mAb recognizes and binds to
5 representative clones of the TH2 cell subpopulation (D10.G4;
DAX), but not clones of the TH1 subtype (AE7; Dorris). For
these experiments, cells were contacted with 3E10 MAb,
preimmune serum (negative control) or a second antiserum
(positive control; referred to as "αTH1 serum" for AE7 and
10 Dorris, and "rat α103 serum" for D10.G4 and DAX). In
contrast, this mAb failed to recognize resting or activated
CD4+ (L-selectin⁻), CD8+, B cells or macrophage cells.

When TH1 cells (AE7, Dorris) were analyzed, the peaks
for 3E10 MAb and the negative preimmune serum exhibited the
15 same very low level of staining as the negative control
preimmune serum. No detectable 103 gene product is present,
therefore, on the surface of the TH1 cells. In contrast,
with TH2 cells (D10.G4, DAX), the 3E10 MAb peak shifted
significantly to the right, demonstrating the presence of 103
20 gene product on the TH2 cell surface. It is noted that for
each clone analyzed, the positive control peak is shifted
well to the right of background levels, as expected.

In addition to the TH2-specific expression pattern
observed in established TH clones as discussed above, 3E10
25 mAb staining and flow cytometry analysis was utilized to
successfully demonstrate that 103 expression dramatically
increases when freshly isolated TH cells are cultured under
conditions that induce TH2 cell polarization, with the
expression being dependent on the degree of differentiation
30 of the TH2 phenotype. Such an increase was not observed
naive CD4+ (L-selection negative) under TH1 cell polarization
conditions (i.e., TH1 effector cells derived from the TH
precursor cells).

As shown in FIG. 26, pretreatment of TH2 recipient mice
35 with 3E10 mAb inhibited the secretion of IL-4, IL-5, IL-6 and
IL-13 by greater than 90%. In particular, analysis of the
cytokine profile in the BAL revealed high levels of IL-4, IL-

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5, IL-6, IL-10 and IL-13 in TH2 recipient OVA challenged mice (closed bars). There was no detectable TH2 cytokines in the BAL fluid of mice that received TH2 cells and were not exposed to ovalbumin. Pretreatment with 3E10 mAb resulted in

5 a dramatic reduction in IL-4, IL-5, IL-6 and IL-13, but had no effect on IL-10 levels in the BAL (open bars). OVA challenge of TH1 recipient mice resulted in high levels of IFN-γ in the BAL fluid (closed bars) that was not inhibited by 3E10 mAb (open bars).

10   These data show that the 103 gene is differentially expressed in a TH2-specific manner, thereby corroborating the results presented in the Example of Section 7, above. In addition, the data demonstrate the feasibility of using antibodies to separate TH2 subpopulation cells away from

15 other cell types, thereby modulating a TH cell subpopulation by changing the number of cells belonging to one TH cell subpopulation relative to that of another TH cell subpopulation.

       An _in vivo_ TH1 and TH2 adoptive transfer model (Cohn, L.

20 et al., 1997, J. Exp. Med. 186:1737-1747) was used to address the role of the 103 gene product in TH cells. In this adoptive transfer animal model, aeroallergen provocation of TH1 or TH2 recipient mice results in TH effector cell migration to the airways and is associated with an intense

25 neutrophilic (TH1) and eosinophilic (TH2) lung mucosal inflammatory response. The model represents an accepted animal model for asthma, a TH2-like disorder. _In situ_ hybridization revealed a marked upregulation of 103 gene mRNA positive cells in lungs from allergen or PBS provoked TH2

30 recipient mice. In marked contrast there was no detectable 103 gene mRNA expression in lungs obtained from either OVA or PBS provoked TH1 recipient mice.

       The animal model was used to investigate whether neutralization of the 103 gene product in vivo also abrogated

35 TH2-mediated pathology. Allergen provocation of mice which had received TH2 cells and control rat Ig resulted in infiltration of lymphocytes and eosinophilic inflammation of

the airways. In vivo administration of 3E10 mAb markedly
suppressed the development of eosinophilic inflammation of
the airways. In particular, eosinophilic inflammation was
assessed, first, by histological analysis of the airway
5 tissue. Second, an analysis of the cellular composition of
the bronchoalveolar lavage fluid (BAL) was performed (FIGS.
27A-27B). No significant reduction in the number of antigen
specific TH2 cells that migrated into the airway interstitium
after allergen challenge was observed.
10   In marked contrast to the effects on TH2 immune
responses, 3E10 treatment did not suppress IFN-γ secretion or
neutrophilic lung inflammation induced by allergen challenge
of TH1 recipient mice. It is also of interest to note that
the anti-103 gene product mAb failed to inhibit IL-10
15 secretion, a cytokine that has been shown to suppress
eosinophil infiltration and prevent IgE mediated mast cell
activation.
     TH2 mediated lung mucosal eosinophilic inflammation is
associated with heightened airway responsiveness to non
20 specific stimuli and is a characteristic feature of bronchial
asthma (Ohashi, Y. et al., 1992, Am. Resp. Dis. 145:1469-76).
To determine whether the 103 gene product is involved in this
physiological consequence of allergen exposure, the degree of
airway constriction induced by the methacholine inhalation
25 was assessed using whole body plethysmography.
     3E10 mAb treatment was, indeed, demonstrated to
attenuate allergen induced heightened airway responsiveness.
In particular, 3E10 mAb treatment suppressed the development
of airway hyperresponsiveness induced by OVA challenge in TH2
30 recipient mice (FIG. 28). TH effects of treatment with 3E10
mAb were comparable to those previously reported using anti-
IL-5 mAbs Wang, L.M., 1992, EMBO 11:4899-4908 and anti-B7-2
mAbs (Tsuyuki, S. et al., 1997, J. Exp. Med. 185:1671-1679).
     The role played by 103 gene in lung inflammation was
35 also investigated in an active immunization model where mice
were injected systemically with antigen and adjuvent prior to
two repeated allergen provocations. As summarized in FIGS.

29A-29B, administration of either 3E10 mAb or 103/Ig fusion
results in a significant reduction in eosinophilic
inflammation, as well as a significant reduction in IL-4 and
IL-5 cytokine levels in the lung, which represent cytokine
5 hallmarks of activated TH2 cell subpopulations. In addition,
3E10 mAb attenuated the induction of OVA specific IgE in the
serum of the active immunization model. Still further, an
approximate 60% reduction in airway hyperresponsiveness was
observed after repeated aerosol challenge after active
10 immunization.

Further, the level of interferon gamma was measured,
which represents a hallmark of TH1 cell subpopulation
activation, and an increase in its level was detected. This
indicates the presence of a relative increase in TH1 cell
15 subpopulation responses.

In addition, animals treated with a soluble fusion
protein containing the extracellular domain of the 103 gene
product fused to an Ig tail (103/Ig fusion). Administration
of the 103/Ig fusion results in significant decrease in
20 hallmark symptoms of asthma. As summarized in FIG. 29B, such
administration results in animals that exhibit a decrease in
eosinophil infiltration into lung airways (this was assessed
by both BAL and histological examination). Likewise,
administration of the 103/Ig fusion resulted in a 50%
25 attenuation in the degree of eosinophilic inflammation of
airways.

Thus, the inhibition of 103 gene function appears to
modulate TH cell subpopulations by decreasing the level
and/or activity of TH2 cells while bringing about a relative
30 increase in the level and/or activity of TH1 cells.

To determine whether signalling through the 103 gene
product directly modifies cytokine production, TH2 effector
cells were activated with plate bound CD3 and CD28. Under
conditions where Fc crosslinking occurred, 3E10 mAb augmented
35 IL-4 and IL-5 secretion in the absence of enhanced
proliferation (FIG. 30). In contrast, CD3/CD28 stimulation
of TH1 cells in the presence of plate bound 3E10MAb failed to

modify IFN-γ secretion. These results indicate that ligation
of the 103 gene product in conjuction with signals delivered
through the CD3/CD28 complex together with CD28 mediated co-
stimulation provide a novel costimulatory signal specific for
5 TH2 effector cells.

     Recently, GATA-3 have been shown to be preferentially
expressed in TH2 cells and suggested to play an important
role in TH2 differentiation (Zheng, W.-P. & Flavell, R.A.,
1997, Cell 89:587-596). Unlike GATA-3, however, the 103 gene
10 product is induced upon CD3/TCR mediated activation and not
during TH2 differentiation from TH0 cells. GATA-3 may be
involved in TH differentiation, while the 103 gene product
may be more involved during activation of TH2 effector cells.
Further, the 103 gene promoter in murine mast cells contains
15 a GATA-3 consensus binding sequence (Gachter, T. et al.,
1996, J. Biol. Chem. 271:124-129), indicating that GATA-3 may
be involved in the TH2 specific expression of the 103 gene.

     In summary, these results provide both in vitro
characterization of 103 gene expression and the 103 gene
20 product, as well as in vivo animal data indicating that the
103 gene product provides a critical signal to TH2 effector
cells and represents a critical regulatory molecule for both
cellular and humoral allergic inflammation. These data
indicate that the 103 gene and/or gene product can be
25 utilized as a novel target for the selective suppression of
TH2 immune responses.

     13. EXAMPLE: THE 200 GENE PRODUCT EXHIBITS A CRITICAL ROLE
          IN THE RESOLUTION OF INJURY FOLLOWING KIDNEY ISCHEMIA AND
30   REPERFUSION

     The Example provided herein presents in vivo data that
the 200 gene product is involved in the recovery from kidney
ischemia injury. In particular, a monoclonal antibody
(96.3.8H7 mAb) generated against the extracellular domain of
35 the murine 200 gene product, and its effect in a surgical
model of recovery from kidney ischemia injury was

investigated.   The anti-200 mAb markedly impaired restoration
of renal function, as determined by several indicators.

    These results, therefore, provide *in vivo* animal data
demonstrating that the 200 gene product provides a critical
5 function in the resolution of injury following kidney
ischemia.   Thus, the 200 gene and its gene product can be
used to aid in the recovery from ischemic injuries such as
stroke, heart attack, acute renal failure, and organ
transplant.
10

13.1.   MATERIALS AND METHODS

    anti-200 mAb: Rat monoclonal antibodies (mAbs) were
generated against the extracellular domain of the mouse 200
gene product.   Recombinant murine 200 gene IgG1 (m200Ig)
15 fusion protein was produced and purified as described in
Section 10.1 above.   Briefly, a DNA sequence containing the
extracellular domain of the mouse 200 gene product was PCR-
amplified and cloned into a vector containing the CD5 signal
sequence and the human IgG1 constant region.   COS cells were
20 transiently transfected using lipofectamine™ (GIBCO)
according to the manufacturer's instructions.   Cells were
cultured in Ultra-Low™ Ig fetal bovine serum (GIBCO) for
approximately one week prior to harvest, and the recombinant
protein was purified by passage over a protein A column.
25   Lu/M rats (Harlan Sprague Dawby, Inc., Indianapolis, IN)
were immunized by subcutaneous injection of 600 mg of
purified recombinant m200Ig fusion protein with 600 μl of
complete Feund's adjuvant.   Rats were boosted twice via
intraperitoneal injections at two week intervals.   The first
30 boost consisted of 500 mg of m200Ig with incomplete Freund's
adjuvant, and the second boost consisted of 500 mg m200Ig in
the absence of Freund's.   Animals were analyzed for
reactivity to the fusion protein by FACS and ELISA 10 days
after the last boost.   Four weeks later, a selected animal
35 which demonstrated positive reactivity to the m200Ig fusion
protein was boosted once more with 500 mg of m200Ig and
sacrificed three days later.   Splenocytes from this animal

were fused with SP2/0 myeloma cells, and resulting clones
were screened and selected to be specific for the murine 200
gene product on the basis of their reactivity to clonal TH2
cell lines (DAX, and D10.G4) and thymocytes from m200
5 transgenic mice versus non-transgenic mice. A single
hybridoma, termed 96.3.8H7, was identified which selectively
reacted to all TH1 cell lines and m200 transgenic thymocytes,
but did not react to TH2 cell lines and non-transgenic
thymocytes.
10

Surgical Methods: Mice were anesthetized with a single
cocktail of Ketamine (200 mg/kg) plux Xylazine (10 mg/kg)
injected intraperitoneally. 1 ml of 0.9% dextrose saline was
also administered subcutaneously. Core body temperature was
15 maintained at around 37 °C. Using a midline abdominal
incision renal arteries and veins were bilaterally occluded
for 32 minutes with microaneurysm clamps, during which time
the abdomen was closed. After the renal pedicle clamps were
removed, the kidneys were observed for an additional five
20 minutes to document color change indicating reflow, and the
incision was sutured. For controls, sham surgery was also
performed on animals, wherein the surgical procedure was
identical except that the microaneurysm clamps were not
aplied.
25   For 200 gene product blockage experiments, mice were
pretreated 24 hours before surgery with 100 µg/mouse of rat
anti-200 monoclonal antibody (200 mAb). In control
experiments, mice were administered equivalent dosages of rat
Ig (RtIg) antibody.
30   Mice were then given antibody at 24 hour intervals
following surgical recovery (4 hours post anesthetic).
        Animals were sacrificed at various periods following
reperfusion, specifically at 12, 24, 48, 72, and 96 hours.
For the later groups (24, 48, 72, and 96 hours post
35 reperfusion) mice were housed in metabolic cages for 24 hours
prior to sacrifice in order to collect urine for protein
analysis as a measure of renal dysfunction. At sacrifice,

the animals were exsanguinated by cardiac puncture under
terminal anesthesia (induced by carbon dioxide in a
precharged chamber) and the kidneys were removed. Serum was
analysed for urea nitrogen and creatinine by Tufts Vetinary

5 Diagnostic Laboratories. One half of each kidney was frozen
in liquid nitrogen for RNA extraction, one quarter was fixed
in 10% buffered formalin for histological assessment by
Hematoxylin and eosin, and the remaining quarter was frozen
for in situ hybridization and immunohistochemical analysis.

10

Kidney Histology: Kidneys were processed, sectioned and
stained with hematoxylin and eosin according to standard
methods. The resulting sections were examined blind and were
assigned an arbitrary score based upon the presence or

15 absence of the following histological parameters: focal
interstitial inflammatory infiltrate, diffuse interstitial
inflammatory infiltrate, glomerular leukocytic infiltrate,
protein deposits in the tubule, presence of apoptotic bodies
in the tubular epithelium, flattened or denuded tubular

20 epithelium, cellular debris in tubules.

13.2. RESULTS

The 200 gene product is similar to the rat adhesion
molecule called KIM-1 (Ichimura et al., 1998, J. Biol. Chem.

25 273:4135), levels of which are increasedin cells after
ischemic/referfusion injury. The role of the 200 gene
product is ischemic injury was investigated in vivo using a
mouse model of acute renal failure. In particular, this
animal model was used to investigate whether neutralization

30 of the 200 gene product in vivo affected tissue repair after
an ischemic injury.

A monoclonal antibody (96.3.8H7 mAb) directed against
the extracellular domain of the 200 gene product was prepared
and characterized, and this antibody was administered to mice

35 24 hours before, and at 24 hour intervals following ischemic
kidney injury. Control animals were adminstered equal
dosages of rat Ig at the same intervals. A second group of

animals underwent sham surgery, described in Section 13.1
above, wherein the animals were not subjected to ischemic
kidney injury.

  Serum creatinine and blood urea nitrogen (BUN) levels
5 were measured in animals sacrificed 24, 48, 72, and 96 hours
after kidney reperfusion. These levels are shown in Table 3
below. Creatinine and blood urea nitrogen levels returned to
basal levels in untreated (+RtIg) mice within 72 hours post
ischemia. However, mice treated with anti-200 mAb (+a200)
10 maintained elevated levels of both blood urea nitrogen (122.5
mg/dl vs. 34.3 mg/dl) and creatinine (0.73 mg/dl vs. 0.3
mg/dl).

15

20

25

30

35

Table 3

	Group	n	Time Point	BUN (mg/dl)	Serum Creatinine (mg/dl)
5	I + RtIg	4	24-hr	39.75 ± 4.77	0.33 ± 0.06
	I + AB	9	24-hr	105.33 ± 15.53	1.02 ± 0.21
	S + RtIg	4	24-hr	20.25 ± 2.93	0.28 ± 0.02
	S + Ab	4	24-hr	29.50 ± 6.08	0.28 ± 0.02
	I + RtIg	4	48-hr	135.00 ± 61.01	1.13 ± 0.49
10	I + AB	11	48-hr	163.82 ± 36.30	1.76 ± 0.48
	S + RtIg	4	48-hr	26.25 ± 3.73	0.30 ± 0.00
	S + Ab	4	48-hr	28.50 ± 3.20	0.28 ± 0.03
	I + RtIg	5	72-hr	37.60 ± 2.23	0.34 ± 0.02
	I + AB	8	72-hr	151.88 ± 54.45	1.09 ± 0.33
15	S + RtIg	3	72-hr	35.00 ± 5.51	0.27 ± 0.03
	S + Ab	4	72-hr	28.50 ± 1.04	0.30 ± 0.00
	I + RtIg	2	96-hr	51.50 ± 23.50	0.35 ± 0.05
	I + AB	5	96-hr	71.80 ± 16.09	0.46 ± 0.08
20	S + Ab	1	96-hr	33.00	0.3

FIG. 31 shows histological cross sections of kidney tisue from untreated (Fig. 31A) and treated (Fig. 31B) rats at 72 hours post reperfusion. Treated mice still exhibited severe tissue damage with flattened tubular epithelial cells, leukocyte infiltration, and tubular cast. The mean histological score was +6 in anti-200 treated mice compared to control mice at 72 hours (Fig. 32).

In summary, these results provide *in vivo* animal data demonstrating that the 200 gene product plays a critical role during recovery from ischemic kidney injury, and can be used as a novel treatment to aid in the recovery from ischemic injuries such as acute renal failure.

14. EXAMPLE: THE 103 GENE PRODUCT IS EXPRESSED IN HUMAN MAST CELLS

The example provided herein presents data demonstrating that the 103 gene product is expressed, in mammalian mast

5 cells. First, Northern analysis showed high levels of 103 gene expression in a human mast cell line. The example also describes the production of monoclonal antibodies which are specific for the human, but not mouse, 103 gene product. FAC staining of the human mast cell line demonstrated binding of

10 these monoclonal antibodies, confirming that the 103 gene product is, indeed, expressed in mast cells.

14.1. MATERIALS AND METHODS

103 fusion proteins: Monoclonal antibodies (mAbs; see

15 below) were generated against the extracellular domain (amino acid residues 18-323) of a human 103 gene product (with a glutamic acid at amino acid residue 78). In particular, the following fusion protein was utilized: a fusion protein that contained, from amino- to carboxy-terminus, a CD5 signal

20 sequence (CD5ss) plus GT residues, the extracellular domain of the 103 gene product described above.

A second Fc fusion protein of the human 103 gene product was constructed, according to the techniques described in Section 10.2 above, to bind ELISA plates for screening. In

25 particular, the protein contain, from amino- to carboxy-terminus, a T075 signal sequence (T075ss) plus QR residues, amino acid residues 20-323 of the human 103 gene product plus a linker amino acid sequence (Ala-Ala-Ala-Asp-Pro) and a human IgG1 constant region.

30 A fusion protein of the mouse 103 gene product was also utilized that contained, from amino- to carboxy-terminus, a CD5 signal sequence plus GT residues, residues 24 (Thr) to 328 (Pro) of the mouse 103 gene product, and human IgG1 constant region.

35 A control fusion protein were also utilized which contained unrelated proteins (T001, a chemokine, or T075, a

tumor necrosis family receptor) fused with a human IgG1 constant region.

The human IgG1 sequence was a in Aruffo et al., 1990, Cell 61:1303.

5

Anti-103 mAB Generation: MAbs were generated in Balb/c mice against the extracellular domain (amino acid residues 18-323) of a human 103 gene product (with a glutamic acid at amino acid residue 78) utilizing the above-described fusion
10 protein, coupled with standard techniques (see Section 5.6, above) for mAb generation, selection and purification.

Splenocytes from animals which showed positive reactivity to the 103 fusion protein were fused with SP2/0 myeloma cells, and the resulting clones were screened and
15 selected to be specific for the human 103 gene product by ELISA and by Biacore (BIAcore, Inc.; Uppsala, Sweden) as described in Fagerstam et al., 1992, J. of Chromatography 597:397-410 and in Kretschmann & Raether, 1968, Z. Naturforschung Teil. A. 23:2135. Twenty-one clones were
20 shown to specifically bind the human 103 gene product (with either alanine or glutamic acid at amino acid residue 78) but not to a mouse 103-gene product Fc fusion protein or to a control Fc fusion protein.

25   Northern Blots: Northern procedures performed in the experiments described in this example were performed as described, above, in Section 6.1.

Flow cytometry analysis of mast cell lines: Cells were
30 analyzed for the expression of gene 103 protein using fluorescence activated cells sorting (FACS) according to standard methods described in Section 12.1, above using anti-mouse IgFITC secondary antibodies.

35

14.2. RESULTS

Northern blot analysis of multiple cells lines showed high levels of the 103 gene in a human mast cell line. Expression of the 103 gene product in this cell line was 5 verified using monoclonal antibodies raised against an Fc fusion protein of the human 103 gene product, as described in Section 14.1, above.

FACS staining of the human mast cell line with the 21 monoclonal antibodies showed staining with 15 of the 21 10 antibodies compared to isotype controls. Five of these 15 antibodies, identified as 1B4, 2O3, 3F7, 3H18, and 10F7, were selected for further analysis. FAC staining with these antibodies was demonstrated to be specifically blocked with an excess of human 103-Fc fusion protein, however, staining 15 was not blocked with control Fc fusion proteins.

In summary, these results provide evidence that the 103 gene product is expressed in a human mast cell line. Accordingly, the 103 gene, its gene product, and compositions derived therefrom (_e.g._, antibodies and other compounds which 20 bind to and/or modulate the expression or activity of the 103 gene or its gene product) may be used not only in the treatment and regulation of TH2 immune disorders such as asthma, but may also be used in the treatment and regulation of mast cell related disorders. Such mast cell related 25 disorders include, but are not limited to, atherosclerosis (see, _e.g._, Metzler and Xu, 1997, _Int. Arch. Allergy Immunol._ _114_:10-14) and myocardial ischemia/reperfusion (see, _e.g._, Frangogiannis _et al._, 1998, _Circulation 98_:699-710).

30

15. DEPOSIT OF MICROORGANISMS

The following microorganisms were deposited with the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, on January 19, 1995 (10-C, 57-E, 105-A, 35 106-H, 161-G, 200-O), March 3, 1995 (_E. coli_ DH10B(Zip)™ containing 200-P) and June 6, 1995 (200-AF, 10-X, 54-C) and assigned the indicated accession numbers:

Microorganism	NRRL Accession No.
10-C	B-21390
57-E	B-21391
105-A	B-21392
106-H	B-21393
161-G	B-21394
200-O	B-21395
E. coli DH10B(Zip)™ containing 200-P cDNA	B-21415
200-AF	B-21457
10-X	B-21455
54-C	B-21456

15   The following microorganisms were deposited with the
American Type Culture Collection (ATCC), 10801 University
Boulevard, Manassas, Virginia, on December 12, 1995 and
assigned the indicated accession numbers:

Microorganism	ATCC Accession No.
E. coli, feht 200C	69967

        The present invention is not to be limited in scope by
the specific embodiments described herein, which are intended
25 as single illustrations of individual aspects of the
invention, and functionally equivalent methods and components
are within the scope of the invention. Indeed, various
modifications of the invention, in addition to those shown
and described herein will become apparent to those skilled in
30 the art from the foregoing description and accompanying
drawings. Such modifications are intended to fall within the
scope of the appended claims.

35

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on pages ___, lines ____ of the description '

A. IDENTIFICATION OF DEPOSIT '

Further deposits are identified on an additional sheet '

Name of depositary institution '

American Type Culture Collection

Address of depositary institution (including postal code and country) '

10801 University Blvd.
Manassas, VA 20110-2209
US

. Date of deposit ' <u>Decemeber 12, 1995</u> Accession Number ' <u>69967</u>

B. ADDITIONAL INDICATIONS ' (leave blank if not applicable). This information is continued on a separate attached sheet

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE ' (if the indications are not all designated States)

D. SEPARATE FURNISHING OF INDICATIONS ' (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later ' (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☑ This sheet was received with the International application when filed (to be checked by the receiving Office)

Jeryl McDowell

PCT/Internat'l Appl Processing Div.

(703) 305-0849

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau '

was   _____
                                       (Authorized Officer)

Form PCT/RO/134 (January 1981)

-215.1-

Form PCT/RO/134 (cont.)

Agricultural Research Culture Collection (NRRL)
International Depositary Authority

1815 N. University Street
Peoria, IL 61604
US

<u>Accession No.</u>	<u>Date of Deposit</u>
B-21390	January 19, 1995
B-21391	January 19, 1995
B-21392	January 19, 1995
B-21393	January 19, 1995
B-21394	January 19, 1995
B-21395	January 19, 1995
B-21415	March 3, 1995
B-21455	June 6, 1995
B-21456	June 6, 1995
B-21457	June 6, 1995

WHAT IS CLAIMED IS:

1.   A method for ameliorating a symptom of an ischemic
disorder or injury in a mammal, comprising administering to
5 the mammal a 200 gene product in an amount effective to
ameliorate the symptom of the ischemic disorder or injury.

2.   A method for ameliorating a symptom of an ischemic
disorder or injury in a mammal, comprising administering to
10 the mammal a nucleic acid molecule encoding a 200 gene
product in an amount effective to ameliorate the symptom of
the ischemic disorder or injury.

3.   A method for ameliorating a symptom of an ischemic
15 disorder or injury in a mammal, comprising administering to
the mammal an antibody directed against a 200 gene product in
an amount effective to ameliorate the symptom of the
disorder.

20   4.   The method of Claim 1, 2, or 3, wherein the
ischemic disorder is ischemic renal disease, or myocardial
ischemia.

5.   The method of Claim 4, wherein the myocardial
25 ischemia is angina pectoris.

6.   The method of Claim 1, 2, or 3 wherein the ischemic
disorder or injury is a infarction.

30   7.   The method of Claim 6, wherein the infarcation is a
myocardial infarction, or a cortical infarction.

8.   The method of Claim 1, 2, or 3, wherein the
ischemic injury is to a transplanted organ.
35

9.   The method of Claim 8, wherein the transplanted
organ is a kidney.

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10. The method of Claim 1, 2, or 3, wherein the 200
gene product is a polypeptide comprising:

    (a) the amino acid sequence of SEQ ID NO:10,

    (b) the amino acid sequence encoded by the nucleotide
5   sequence of SEQ ID NO:8,

    (c) the amino acid sequence encoded by the cDNA insert
of the clone *E. coli* DH10B(Zip)™ containing 200-P
(NRRL Accession No. B-21415), 200-AF (NRRL
Accession No. B-21457), or 200-O (NRRL Accession
10   No. B-21395),

    (d) the amino acid sequence of SEQ ID NO:24,

    (e) the amino acid sequence encoded by the nucleotide
sequence of SEQ ID NO:37, or

    (f) the amino acid sequence encoded by the cDNA insert
15   of the clone feht200C (ATCC Accession No. 69967).

11. The method of Claim 1, 2, or 3, wherein the 200
gene product is a polypeptide encoded by a nucleic acid
molecule which hybridizes under highly stringent conditions
20 to the complement of:

    (a) a nucleic acid molecule which encodes the amino
acid sequence of SEQ ID NO:10;

    (b) a nucleic acid molecule comprising the nucleotide
sequence of SEQ ID NO:8,

25   (c) the cDNA sequence contained in the clone *E. coli*
DH10B(Zip)™ containing 200-P (NRRL Accession No. B-
21415), 200-AF (NRRL Accession No. B-21457), or
200-O (NRRL Accession No. B-21395),

    (d) to the complement of a nucleic acid molecule which
30   encodes the amino acid sequence of SEQ ID NO:24,

    (e) to the complement of the nucleotide sequence of SEQ
ID NO:37, or

    (f) to the complement of the cDNA sequence contained in
the clone feht200C (ATCC Accession No. 69967).

35

WO 00/73498

12. The method of Claim 2 wherein the nucleic acid molecule encoding a gene 200 product comprises:

    (a)   a nucleotide sequence which encodes the amino acid sequence of SEQ ID NO:10,

5   (b)   the nucleotide sequence of SEQ ID NO:8,

    (c)   the nucleotide sequence of the cDNA insert of the clone *E. coli* DH10B(Zip)™ containing 200-P (NRRL Accession No. B-21415), 200-AF (NRRL Accession No. B-21457), or 200-O (NRRL Accession No. B-21395),

10   (d)   a nucleotide sequence which encodes the amino acid sequence of SEQ ID NO:24,

    (e)   the nucleotide sequence of SEQ ID NO:37, or

    (f)   the nucleotide sequence of the cDNA insert of the clone feht200c (ATCC Accession No. 69967).

15

13. The method of Claim 1 wherein said administering of the 200 gene product is parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, or intralesional.

20

14. The method of Claim 13, wherein the intralesional administration comprises perfusing or contacting a graft or organ with the 200 gene product before transplant.

25   15. The method of Claim 2 wherein said administering of the nucleic acid is parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, or intralesional.

30   16. The method of Claim 15, wherein the intralesional administration comprises perfusing or contacting a graft or organ with the nucleic acid before transplant.

17. The method of Claim 3 wherein said administering of 35 the antibody is parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, or intralesional.

18. The method of Claim 17, wherein the intralesional administration comprises perfusing or contacting a graft or organ with the antibody before transplant.

5   19. The method of Claim 3, wherein the amount of the antibody administered is from about 1 µg/kg to about 100 mg/kg.

20. The method of Claim 19, wherein the amount of the
10 antibody administered is from about 1 µg/kg to about 15 mg/kg.

21. The method of Claim 20, wherein the amount of the antibody administered is from about 0.1 mg/kg to about 2.0
15 mg/kg.

20

25

30

35
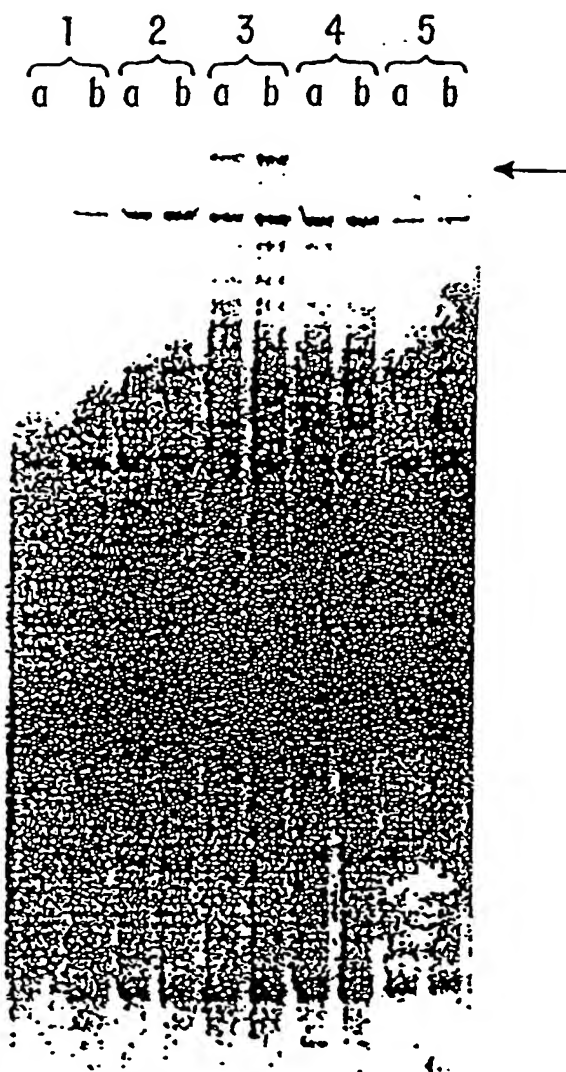
FIG. 1

```
         10         20         30         40         50         60 ·
CTGGTGAGGG GGATCTACAA CTTGTTCGGT TAAAGAAAAA AGCAACAGCC AACAGAAATG  60
TGGTTATCCT TCACCTACCT AAAAAGGGAG ATGATGTGAA ACCAGGAACC AGATGCCGAG 120
TAGCAGGATG GGGGAGATTT GGCAATAAGT CAGCTCCCTC TGAAACTCTG AGAGAAGTCA 180
ACATCACTGT CATAGACAGA AAAATCTGCA ATGATGAAAA ACACTATAAT TTTCATCCTG 240
TAATTGGTCT·AAACATGATT TGGGCAGGGG ACCTCCCCGG CGGAAAGGAC TCCTGCAATG 300
GGGATTCTGG CAGCCCTCTC CTATGTGATT GGTATTTGGG AAGCATCACC TCCTTTT     357
```

FIG. 2

```
         10         20         30         40         50         60
TTAGCGCCAT TGCCATAGAG AGACCTCAGC CATCAATCAC TAGCACATGA TTGACAGACA  60
·GAGAATGGGA CTTTGGGCTT TGGCAATTCT GACACTTCCC ATGTATTTGA CAGTTACGGA 120
GGGCAGTAAA TCGTCCTGGG GTCTGGAAAA TGAGGCTTTA ATTGTGAGAT GCCCCCAAAG 180
AGGACGCTCG ACTTATCCTG TGGAATGGTA TTACTCAGAT ACAAATGAAA GTATTCCTAC 240
CCAAAAAAAA AAAAA                                                  255
```

FIG. 4A
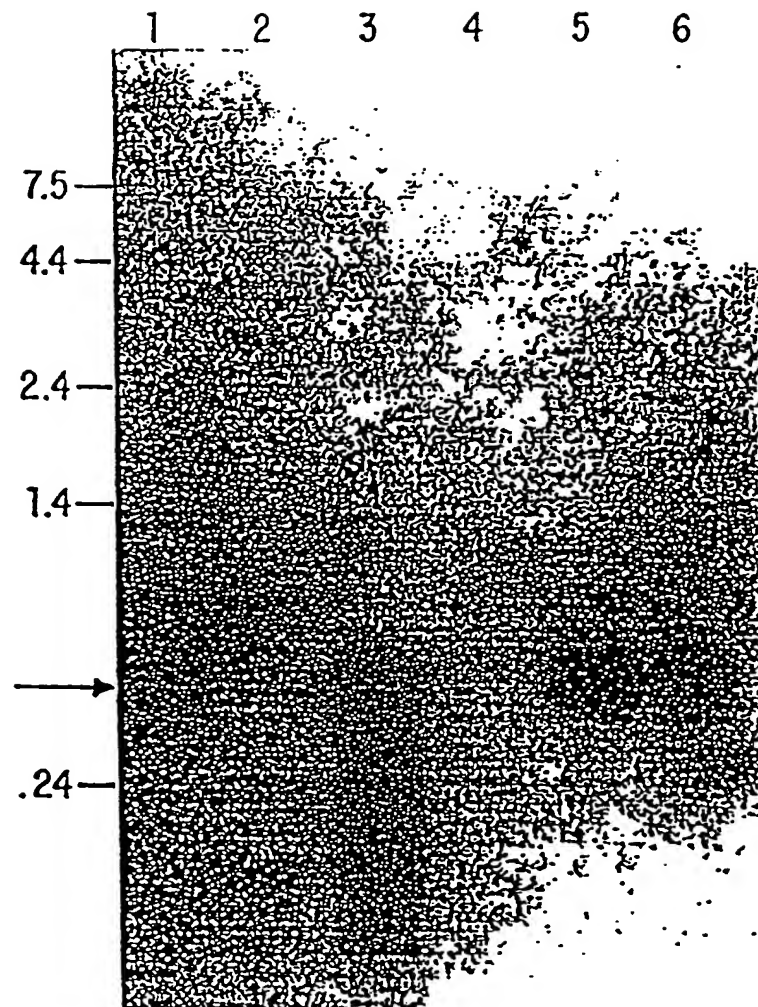
FIG. 3
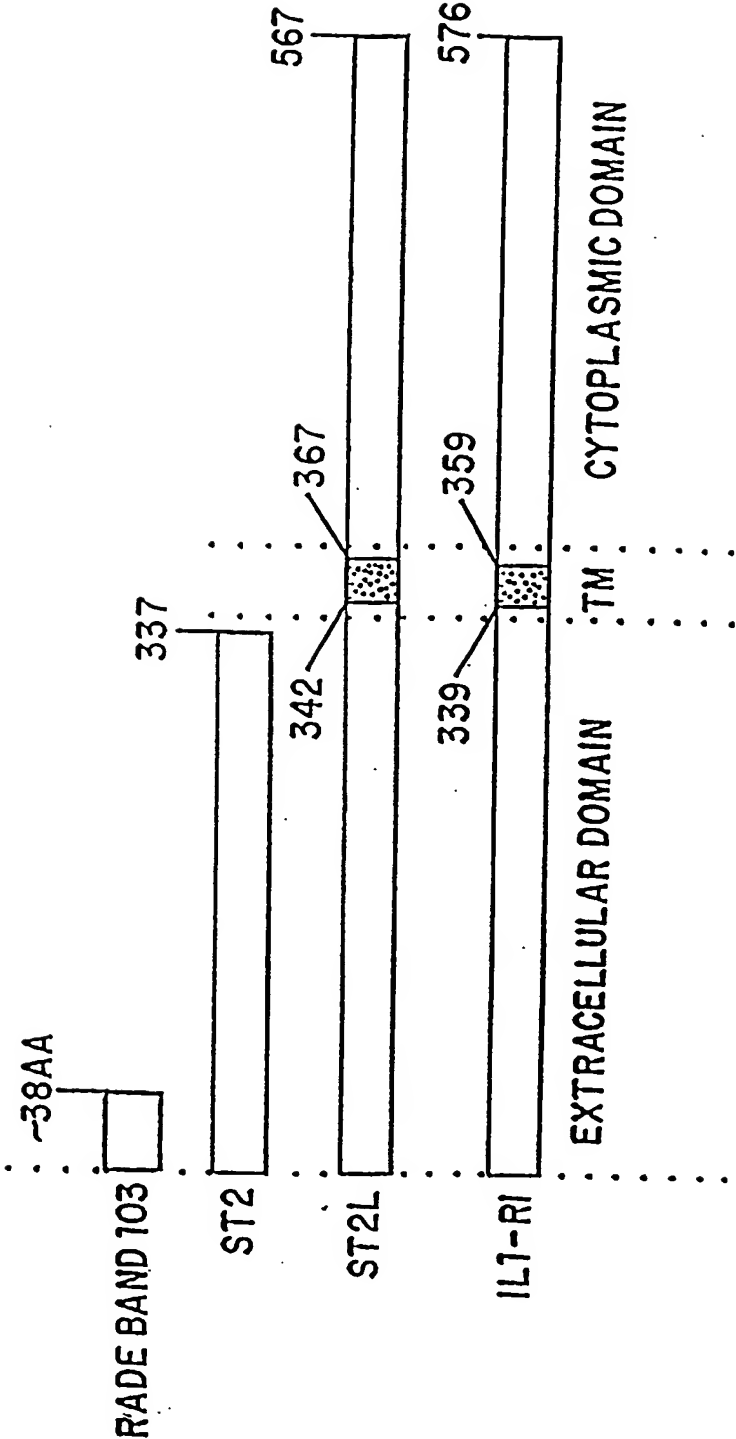
FIG. 4B

```
  1 atgattgaca gacagagaat gggactttgg gctttggcaa ttctgacact tcccatgtat
 61 ttgacagtta cggaggggcag taaatcgtcc tggggtctgg aaaatgaggc tttaattgtg
121 agatgccccc aaagaggacg ctcgacttat cctgtggaat ggtattactc agatacaaat
181 gaaagtattc ctactcaaaa aagaaatcgg atctttgtct caagagatcg tctgaagttt
241 ctaccagcca gagtcgaaga ctctgggatt tatgcttgtg ttatcagaag ccccaacttg
301 aataagactg gatacttgaa tgtcaccata cataaaaagc cgccaagctg caatatccct
361 gattatttga tgtactcgac agtacgtgga tcagataaaa atttcaagat aagctgtcca
421 acaattgacc tgtataattg gacagcacct gttcagtggt ttaagaactg caaagctctc
481 caagagccaa ggttcagggc acacaggtcc tacttgttca ttgacaacgt gactcatgat
541 gatgaaggtg actacacttg tcaattcaca cacgcggaga atggaaccaa ctacatcgtg
601 acggccacca gatcattcac agttgaagaa aaaggctttt ctatgtttcc agtaattaca
661 aatcctccat acaaccacac aatggaagtg gaaataggaa aaccagcaag tattgcctgt
721 tcagcttgct ttggcaaagg ctctcacttc ttggctgatg tcctgtggca gattaacaaa
781 acagtagttg gaaattttgg tgaagcaaga attcagaag aggaaggtcg aaatgaaagt
841 tccagcaatg acatggattg tttaacctca gtgttaagga taactggtgt gacagaaaag
901 gacctgtccc tggaatatga ctgtctggcc ctgaaccttc atggcatgat aaggcacacc
961 ataaggctga gaaggaaaca accaagtaag gagtgtccct cacacattgc t
```

FIG. 4C

MIDRQRMGLWALAILTLPMYLTVTEGSKSSWGLENEALIVRCPQRGRSTYPVEWYYSD
TNESIPTQKRNRIFVSRDRLKFLPARVEDSGIYACVIRSPNLNKTGYLNVTIHKKPPSCNIP
DYLMYSTVRGSDKNFKITCPTIDLYNWTAPVQWFKNCKALQEPRFRAHRSYLFIDNVTH
DDEGDYTCQFTHAENGTNYIVTATRSFTVEEKGFSMFPVITNPPYNHTMEVEIGKPASIA
CSACFGKGSHFLADVLWQINKTVVGNFGEARIQEEEGRNESSSNDMDCLTSVLRITGVT
EKDLSLEYDCLALNLHGMIRHTIRLRRKQPSKECPSHIA

FIG. 4D

```
ATGATTGACA GACAGAGAAT GGGACTTTGG GCTTTGGCAA TTCTGACACT TCCCATGTAT     60
TTGACAGTTA CGGAGGGCAG TAAATCGTCC TGGGGTCTGG AAAATGAGGC TTTAATTGTG    120
AGATGCCCCC AAAGAGGACG CTCGACTTAT CCTGTGGAAT GGTATTACTC AGATACAAAT    180
GAAAGTATTC CTACTCAAAA AAGAAATCGG ATCTTTGTCT CAAGAGATCG TCTGAAGTTT    240
CTACCAGCCA GAGTGGAAGA CTCTGGGATT TATGCTTGTG TTATCAGAAG CCCCAACTTG    300
AATAAGACTG GATACTTGAA TGTCACCATA CATAAAAAGC CGCCAAGCTG CAATATCCCT    360
GATTATTTGA TGTACTCGAC AGTACGTGGA TCAGATAAAA ATTTCAAGAT AACGTGTCCA    420
ACAATTGACC TGTATAATTG GACAGCACCT GTTCAGTGGT TTAAGAACTG CAAAGCTCTC    480
CAAGAGCCAA GGTTCAGGGC ACACAGGTCC TACTTGTTCA TTGACAACGT GACTCATGAT    540
GATGAAGGTG ACTACACTTG TCAATTCACA CACGCGGAGA ATGGAACCAA CTACATCGTG    600
ACGGCCACCA GATCATTCAC AGTTGAAGAA AAAGGCTTTT CTATGTTTCC AGTAATTACA    660
AATCCTCCAT ACAACCACAC AATGGAAGTG AAATAGGAA AACCAGCAAG TATTGCCTGT    720
TCAGCTTGCT TTGGCAAAGG CTCTCACTTC TTGGCTGATG TCCTGTGGCA GATTAACAAA    780
ACAGTAGTTG GAAATTTTGG TGAAGCAAGA ATTCAAGAAG AGGAAGGTCG AAATGAAAGT    840
TCCAGCAATG ACATGGATTG TTTAACCTCA GTGTTAAGGA TAACTGGTGT GACAGAAAAG    900
GACCTGTCCC TGGAATATGA CTGTCTGGCC CTGAACCTTC ATGGCATGAT AAGGCACACC    960
ATAAGGCTGA GAAGGAAACA ACCAATTGAT CACCGAAGCA TCTACTACAT AGTTGCTGGA   1020
TGTAGTTTAT TGCTAATGTT TATCAATGTC TTGGTGATAG TCTTAAAAGT GTTCTGGATT   1080
GAGGTTGCTC TGTTCTGGAG AGATATAGTG ACACCTTACA AAACCCGGAA CGATGGCAAG   1140
CTCTACGATG CGTACATCAT TTACCCTCGG GTCTTCCGGG GCAGCGCGGC GGGAACCCAC   1200
TCTGTGGAGT ACTTTGTTCA CCACACTCTG CCCGACGTTC TTGAAAATAA ATGTGGCTAC   1260
AAATTGTGCA TTTATGGGAG AGACCTGTTA CCTGGGCAAG ATGCAGCCAC CGTGGTGGAA   1320
AGCAGTATCC AGAATAGCAG AAGACAGGTG TTTGTTCTGG CCCCTCACAT GATGCACAGC   1380
AAGGAATTTG CCTACGAGCA GGAGATTGCT CTGCACAGCG CCCTCATCCA GAACAACTCC   1440
AAGGTGATTC TTATTGAAAT GGAGCCTCTG GGTGAGGCAA GCCGACTACA GGTTGGGGAC   1500
CTGCAAGATT CTCTCCAGCA TCTTGTGAAA ATTCAGGGGA CCATCAAGTG GAGGGAAGAT   1560
CATGTGGCCG ACAAGCAGTC TCTAAGTTCC AAATTCTGGA AGCATGTGAG GTACCAAATG   1620
CCAGTGCCAG AAAGAGCCTC CAAGACGGCA TCTGTTGCGG CTCCGTTGAG TGGCAAGGCA   1680
TGCTTAGACC TGAAACACTT TTGA                                          1704
```

FIG. 4E

MIDRQRMGLWALAILTLPMYLTVTEGSKSSWGLENEALIVRCPQRGRSTYPVEWYYSD
TNESIPTQKRNRIFVSRDRLKFLPARVEDSGIYACVIRSPNLNKTGYLNVTIHKKPPSCNIP
DYLMYSTVRGSDKNFKITCPTIDLYNWTAPVQWFKNCKALQEPRFRAHRSYLFIDNVTH
DDEGDYTCQFTHAENGTNYIVTATRSFTVEEKGFSMFPVITNPPYNHTMEVEIGKPASIA
CSACFGKGSHFLADVLWQINKTVVGNFGEARIQEEEGRNESSSNDMDCLTSVLRITGVT
EKDLSLEYDCLALNLHGMIRHTIRLRRKQPIDHRSIYYIVAGCSLLLMFINVLVIVLKVFW
IEVALFWRDIVTPYKTRNDGKLYDAYIIYPRVFRGSAAGTHSVEYFVHHTLPDVLENKC
GYKLCIYGRDLLPGQDAATVVESSIQNSRRQVFVLAPHMMHSKEFAYEQEIALHSALIQ
NNSKVILIEMEPLGEASRLQVGDLQDSLQHLVKIQGTIKWREDHVADKQSLSSKFWKHV
RYQMPVPERASKTASVAAPLSGKACLDLKHF

FIG. 4F

```
   1 atctcaacaa cgagttacca atacttgctc ttgattgata aacagaatgg ggttttggat
  61 cttagcaatt ctcacaattc tcatgtattc cacagcagca aagtttagta aacaatcatg
 121 gggcctggaa aatgaggctt taattgtaag atgtcctaga caaggaaaac ctagttacac
 181 cgtggattgg tattactcac aaacaaacaa aagtattccc actcaggaaa gaaatcgtgt
 241 gtttgcctca ggccaacttc tgaagtttct accagctgaa gttgctgatt ctggtattta
 301 tacctgtatt gtcagaagtc ccacattcaa taggactgga tatgcgaatg tcaccatata
 361 taaaaaacaa tcagattgca atgttccaga ttatttgatg tattcaacag tatctggatc
 421 agaaaaaaat tccaaaattt attgtcctac cattgaccte tacaactgga cagcacctct
 481 tgagtggttt aagaattgtc aggctcttca aggatcaagg tacagggcgc acaagtcatt
 541 tttggtcatt gataatgtga tgactgagga cgcaggtgat tacacctgta aatttataca
 601 caatgaaaat ggagccaatt atagtgtgac ggcgaccagg tccttcacgg tcaaggatga
 661 gcaaggcttt tctctgtttc cagtaatcgg agcccctgca caaaatgaaa taaaggaagt ·
 721 ggaaattgga aaaaacgcaa acctaacttg ctctgcttgt tttggaaaag gcactcagtt
 781 cttggctgcc gtcctgtggc agcttaatgg aacaaaaatt acagactttg gtgaaccaag
 841 aattcaacaa gaggaagggc aaaatcaaag tttcagcaat gggctggctt gtctagacat
 901 ggttttaaga atagctgacg tgaaggaaga ggatttattg ctgcagtacg actgtctggc
 961 cctgaatttg catggcttga gaaggcacac cgtaagacta agtaggaaaa atccaagtaa
1021 ggagtgtttc tgagactttg atcacctgaa ctttctctag caagtgtaag cagaatggag
1081 tgtggttcca agagatccat caagacaatg ggaatggcct gtgccataaa atgtgcttct
1141 cttcttcggg atgttgtttg ctgtctgatc tttgtagact gttcctgttt gctgggagct
1201 tctctgctgc ttaaattgtt cgtcctcccc cactccctcc tatcgttggt ttgtctagaa
1261 cactcagctg cttctttggt catccttgtt ttctaacttt atgaactccc tctgtgtcac
1321 tgtatgtgaa aggaaatgca ccaacaaccg aaaactg
```

FIG. 4G

MGFWILAILTILMYSTAAKFSKQSWGLENEALIVRCPRQGKPSYTVDWYYSQTNKSIPT
QERNRVFASGQLLKFLPAEVADSGIYTCIVRSPTFNRTGYANVTIYKKQSDCNVPDYLM
YSTVSGSEKNSKIYCPTIDLYNWTAPLEWFKNCQALQGSRYRAHKSFLVIDNVMTEDAG
DYTCKFIHNENGANYSVTATRSFTVKDEQGFSLFPVIGAPAQNEIKEVEIGKNANLTCSA
CFGKGTQFLAAVLWQLNGTKITDFGEPRIQQEEGQNQSFSNGLACLDMVLRIADVKEED
LLLQYDCLALNLHGLRRHTVRLSRKNPSKECF

FIG. 4H
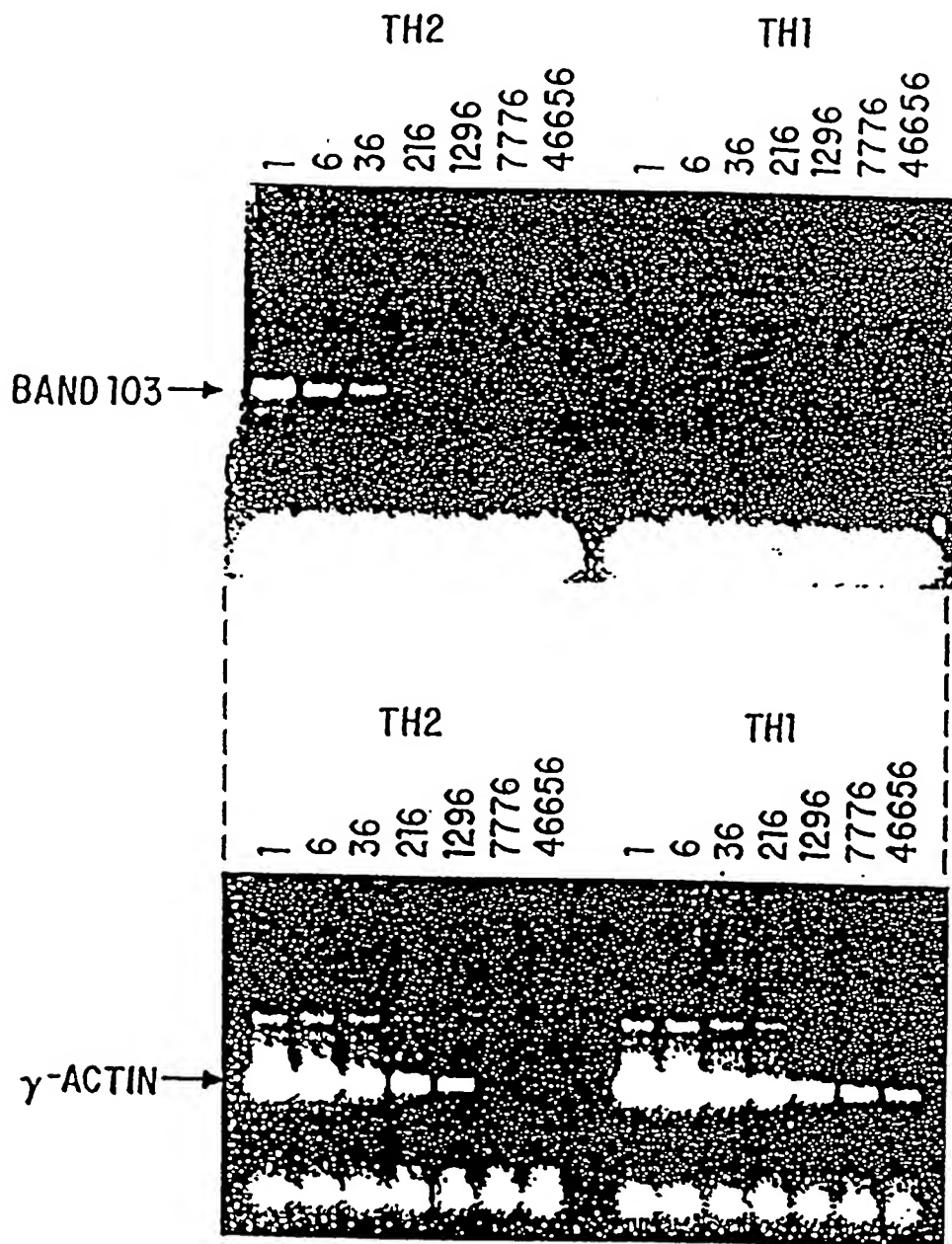
FIG. 5
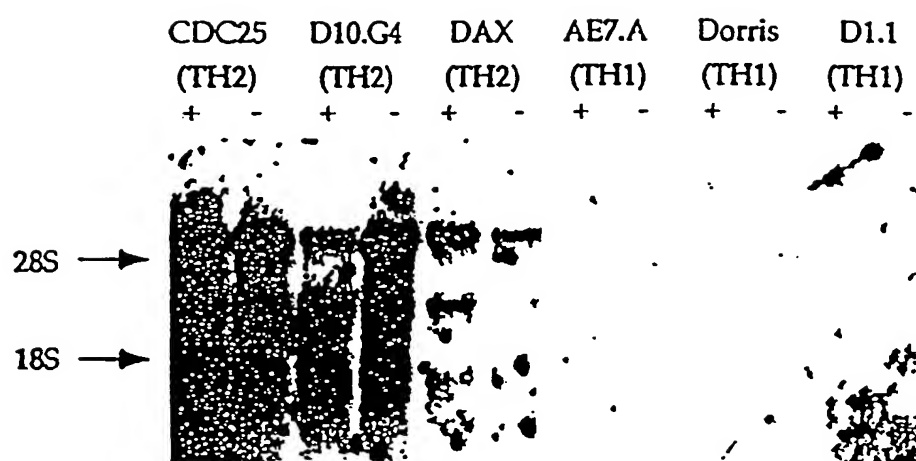
FIG. 6
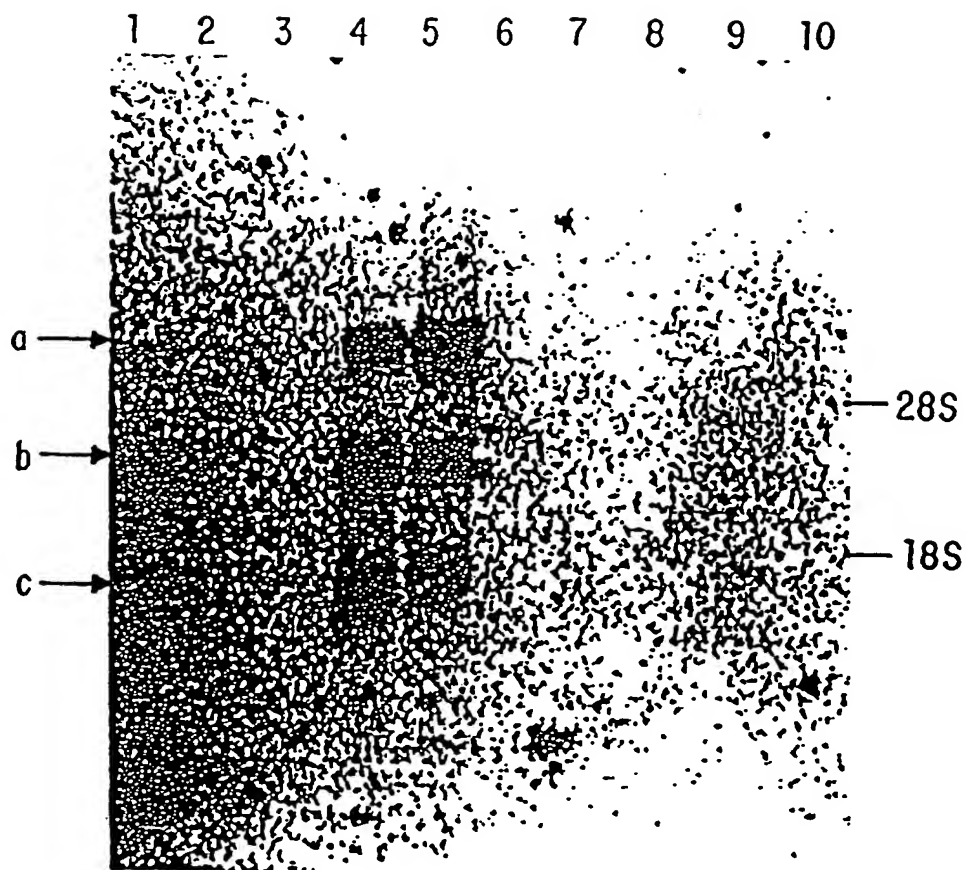
FIG. 7
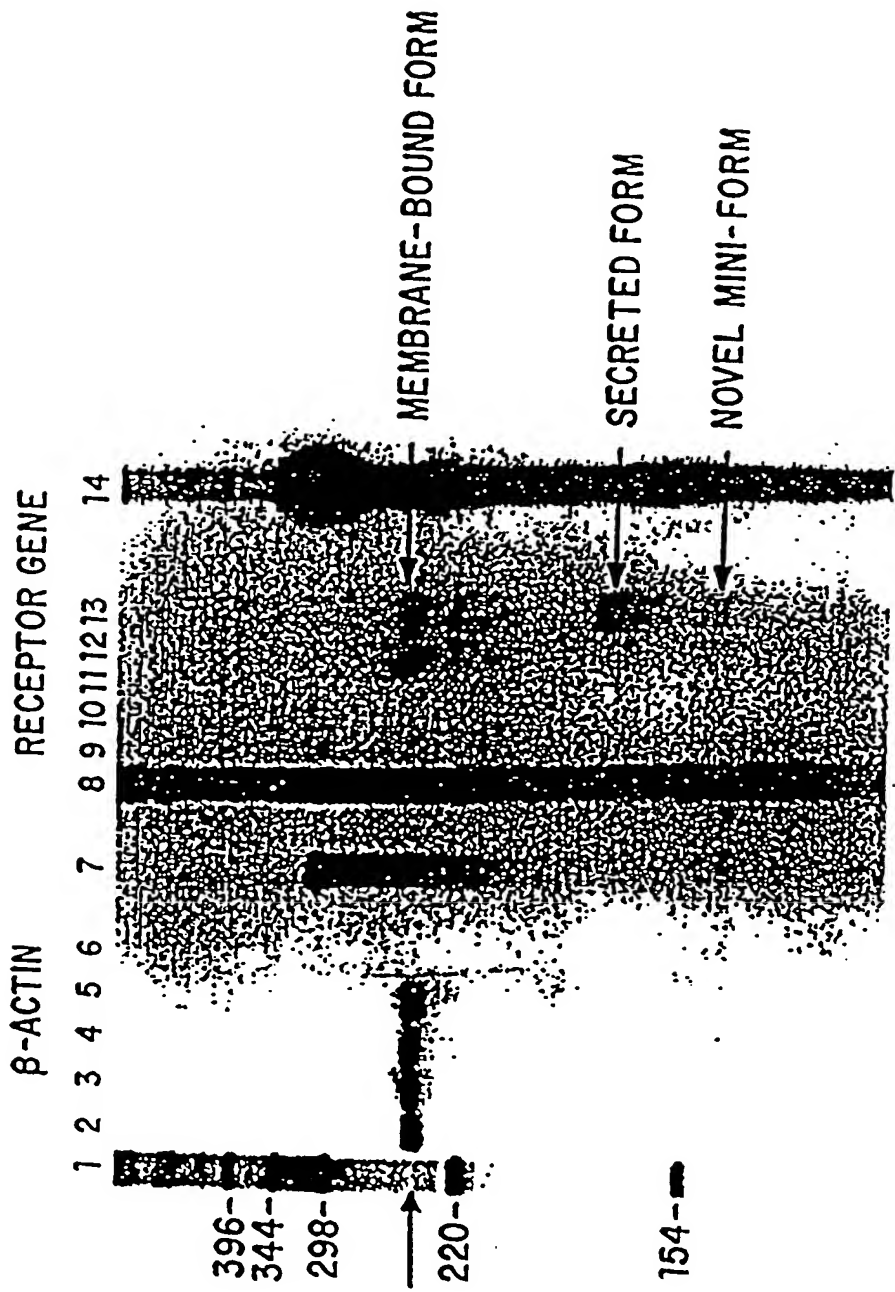
FIG. 8

```
                                                                              CC    2

GGGTCGACCACGCGTCCGAGCCTCCTCAGTCAAGAGAAGCATCCCTCCAGAAACAGGGAAACATGACACTTTTGAAAG   81

AATGCCAAACGGGCGGTGAAATAAAAACAGAGCATTCCCATTTGCACCGACCAATCTCCTGTAAGATTCAAAA        160

GGGCAAGCAAGAGGCGGTGACCGTTCACGAAAGCTAAAATCCCATGCTATTGAACATGAAGACTTCTGATGCTTAAATC  239

TCATTAACTGCTTTAAGTCACTCCCAGGAGCTTGGATCCCAACTTCTAGCAGTAATAGTCTGTGTAAAAAAAAAAAAAA  318

AATCAGTGCTACAACCACTCTCTAAATGCATGGATGAACTCATCAGAACATCAAAACCCAAGGAAACCCTAAGAGAGAAG 397

AATTCTAATAAAAGAATTTTACATTGAAAACTTACAGGCAAGGTCCCTTTCCCTGCTGACAGCCTAAGAAGTGATGT    476

                 M   A   M   N   S   M   C   I   E   E   Q   R   H   L   E     15
AACTGCCACTGTGAAGACC ATG GCG ATG AAC AGC ATG TGC ATT GAA GAG CAG CAG CGC CAC CTC GAA  540

 H   Y   L   F   P   V   V   Y   I   I   V   F   I   V   S   V   P   A   N   I     35
CAC TAT TTG TTC CCG GTG GTC TAC ATA ATT GTG TTT ATA GTC AGC GTC CCA GCC AAC ATC  600

 G   S   L   C   V   S   F   L   Q   A   K   K   E   N   E   L   G   I   Y   L     55
GGA TCT TTA TGC GTA TCC TTT CTT CTG CAA GCG AAG AAG GAA AAT GAG CTA GGG ATT TAC CTC  660

 F   S   L   S   D   L   L   Y   A   L   T   L   P   L   W   I   N   Y           75
TTC AGT CTG TCC CTG TCA GAC CTG CTG TAT GCG CTG ACT CTG CCC CTC TGG ATC AAT TAC  720
```

FIG. 9A

```
T   W   N   K   D   N   W   T   F   S   P   T   L   C   K   G   S   V   F   F    95
ACT TGG AAT AAA GAC AAC TGG ACT TTC TCT CCC ACC TTG TGC AAA GGA AGC GTT TTC TTC  780

T   Y   M   N   F   Y   S   S   T   A   F   L   I   C   I   A   L   D   R   Y   115
ACC TAC ATG AAC TTT TAC AGC ACG GCG TTC CTC ACT TGC ATT GCC CTG GAC CGC TAT   840

L   A   V   V   Y   P   L   K   F   S   F   L   R   R   T   R   R   F   A   F   I  135
TTA GCA GTC GTC TAC CCT CTG AAG TTT TCC TTC CTA AGA ACG AGA AGA TTC GCG TTT ATT  900

T   S   L   S   I   W   I   L   E   S   F   F   N   S   M   L   W   K   D   155
ACC AGC CTC ATC TGG ATA TTA GAG TCC TTC TTT AAC TCT ATG CTT CTG TGG AAA GAT   960

E   T   S   V   E   Y   C   D   S   D   K   S   N   F   T   L   C   Y   D   K   175
GAA ACG AGT GTT GAA TAT TGT GAC TCG GAC AAA TCT AAT TTC ACT CTC TGC TAT GAC AAA  1020

Y   P   L   E   K   W   Q   I   N   L   N   L   F   R   T   C   M   G   Y   A   195
TAC CCT CTG GAG AAA TGG CAG ATA AAC CTC AAC CTG TTT CGG ACG TGC ATG GGC TAC GCA  1080

I   P   L   I   T   I   M   I   C   N   H   K   V   Y   R   A   V   R   H   N   215
ATA CCC TTG ATC ACC ATC ATG ATC TGC AAC CAT AAA GTC TAC CGA GCT GTG CGG CAC AAC  1140

Q   A   T   E   N   S   E   K   R   R   I   K   L   L   A   S   I   T   L   235
CAA GCC ACG GAA AAC AGC GAG AAG AGA ATC ATA AAG TTG CTT GCT AGC ATC ACG TTG  1200

T   F   V   L   C   F   T   P   F   H   V   M   V   L   I   R   C   V   L   E   255
ACT TTC GTC CTA TGC TTC ACC CCC TTC CAC GTG ATG GTG CTC ATC CGC TGC GTT TTA GAG  1260
```

FIG. 9B

```
       R   D   M   N   V   N   D   K   S   G   W   Q   T   F   T   V   Y   R   V   T   275
       CGC GAC ATG AAC GTC AAT GAC AAG TCT GGA TGG CAG ACG TTT ACG GTG TAC AGA GTC ACA 1320

       V   A   L   T   S   L   N   C   V   A   D   P   I   L   Y   C   F   V   T   E   295
       GTA GCC CTG ACG AGT CTA AAC TGT GTT GCC GAT CCC ATT CTG TAC TGC TTT GTG ACT GAG 1380

       T   G   R   A   D   M   W   N   I   L   K   L   C   T   R   K   H   N   R   H   315
       ACG GGG AGA GCT GAT ATG TGG AAC ATA TTA AAA TTG TGT ACT AGG AAA CAC AAT AGA CAC 1440

       Q   G   K   K   R   D   I   L   S   V   S   T   R   D   A   V   E   L   E   I   335
       CAA GGG AAA AAA AGG GAC ATA CTT TCT GTG TCC ACA AGA GAT GCT GTA GAA TTA GAG ATT 1500

       I   D   *                                                                     338
       ATA GAC TAA GAGGTGGAGGGCAGGTTAAGTTACACATGGTATTATTTAATGAACTTACATTTTGGAAAAGAAATCTGG 1576

       CATAGTAGAACCCAGTGGAAATAGTTTGAAGGTACATTGTATGACTCCTATGTTGGCTTATTAAGTAAGGTATAGAAA 1655

       TGTATTATCTTGTATGTATTCTAATGACTAGGCATCATTGTTTTAGTACCAATTCTCTTTGCCTCTATGTTATAACCCC 1734

       TAAGAAGCACGCGGGACTGTTCGTCTTAAATCAGTGGCCATTCTATCTGACTACTATGACTTTTGTTGTTGTTCTGC 1813

       TTTGGGTTTTCAGTCTGCCTGCATCAGTCTTCTCCCTGTATACGTCTGTCTTCAACAAATGTAAGGACTAAATACCCC 1892
```

FIG. 9C

TCCCGATCACATCCATTATCAAGGATTTGAAGGCCACTCCATGTACTGGGTTATAAAGAAATGTTCTCATGAACTTTCA 1971

TGAAGTTTACATACCTTTGGGGATCTAGTCACCGAGTCACATAAAGTAAAGTAAATGGAAAAAAAAAAAAAAAAAAA 2050

AGGGC   2055

FIG. 9D
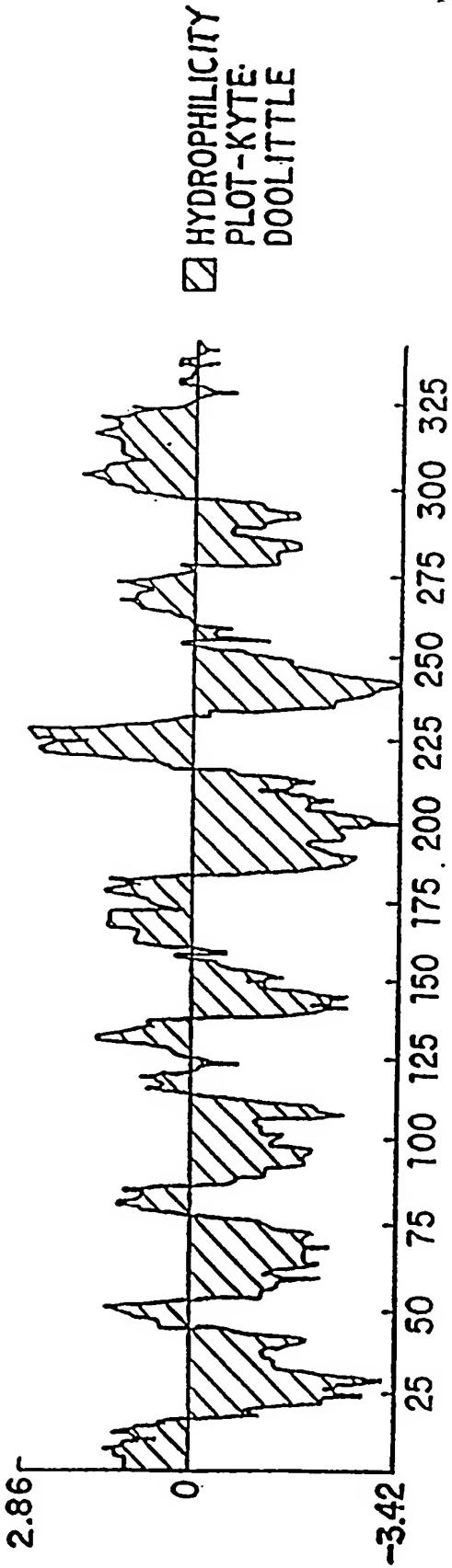
FIG.10A
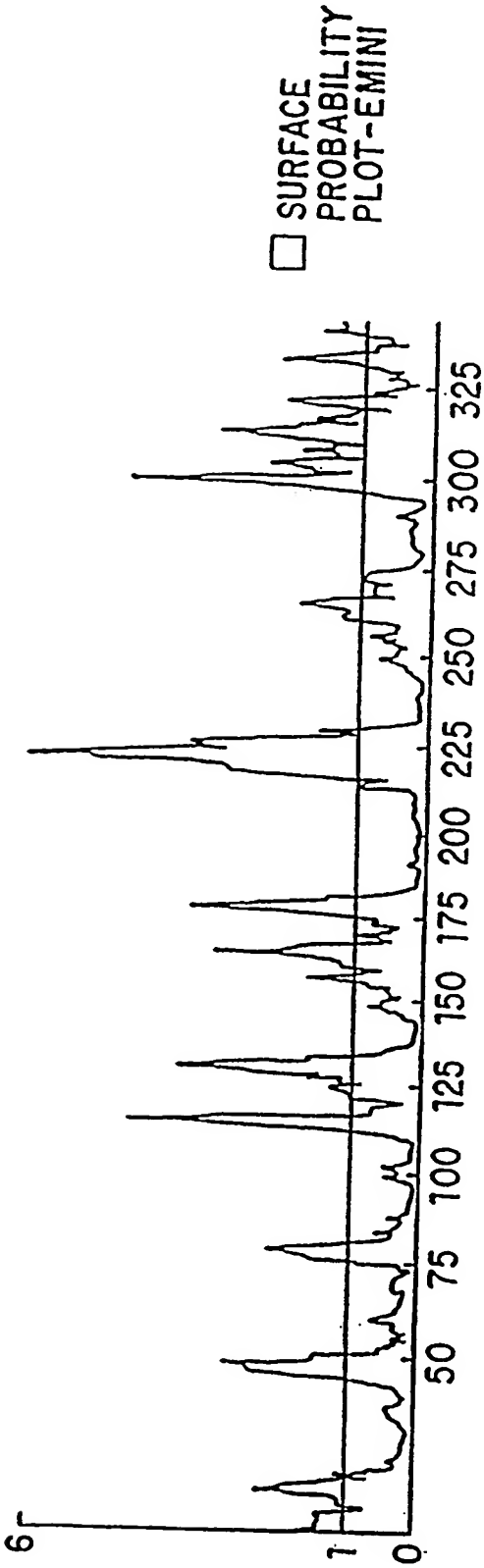
FIG.10B
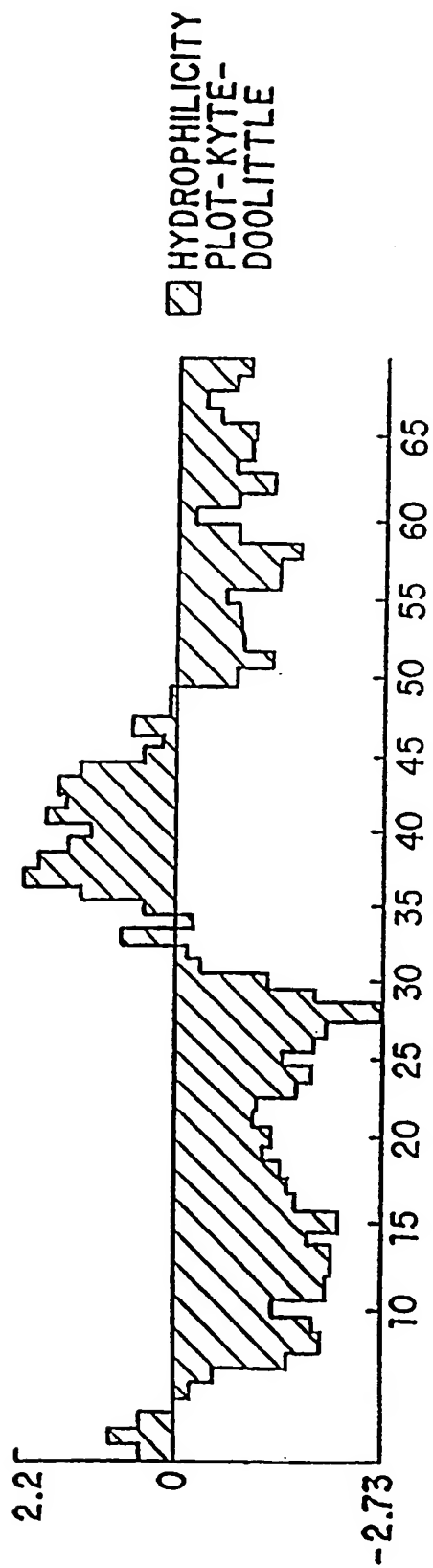
FIG. 10C
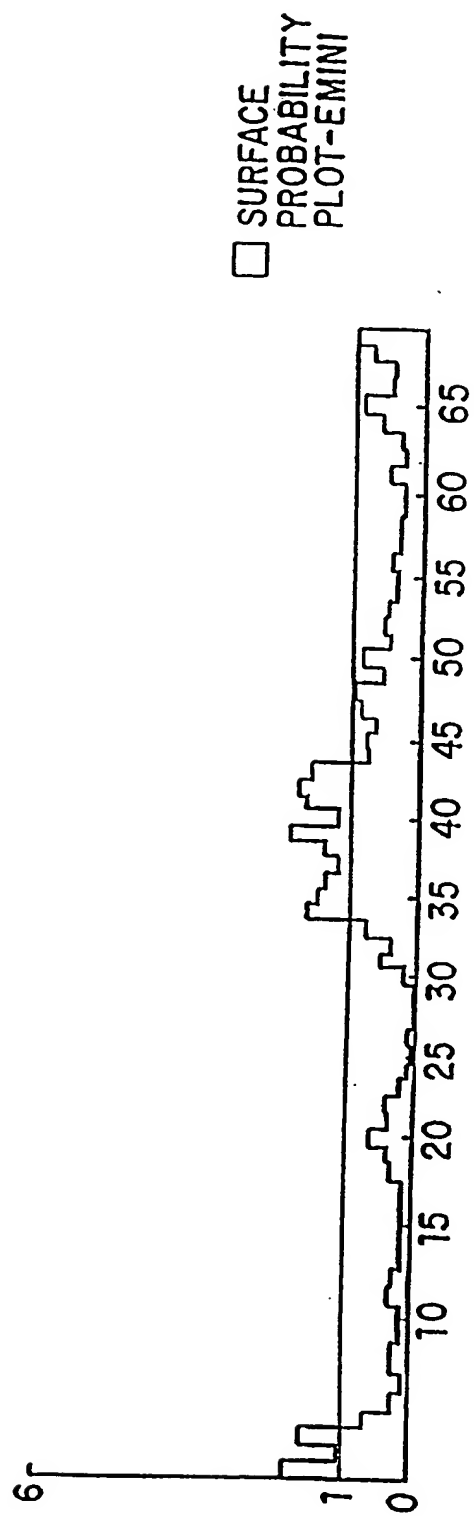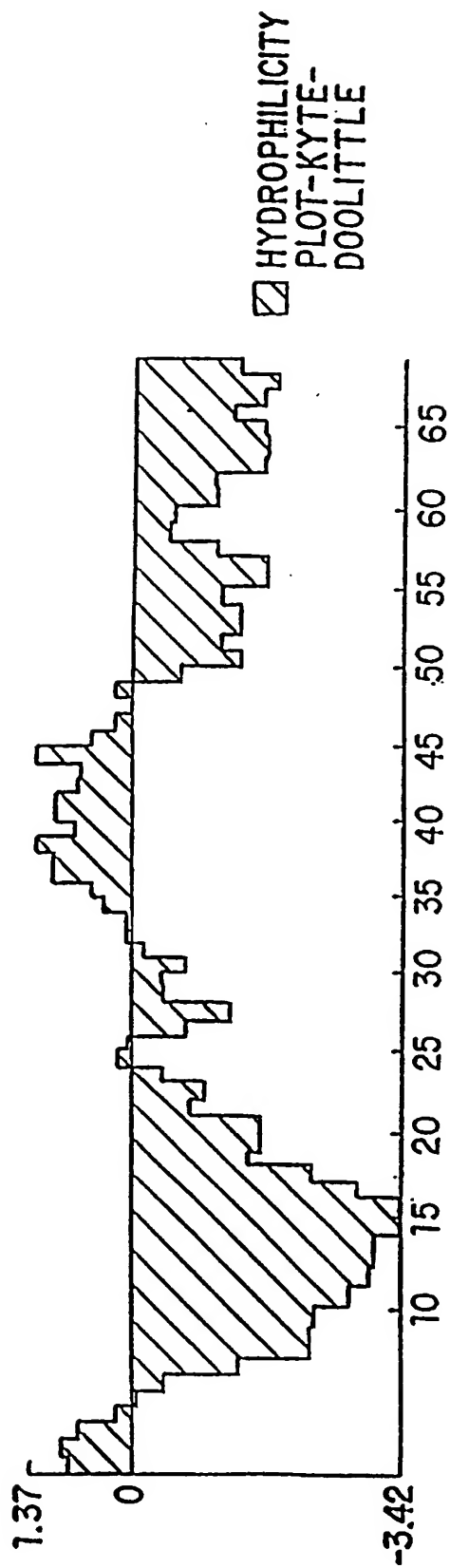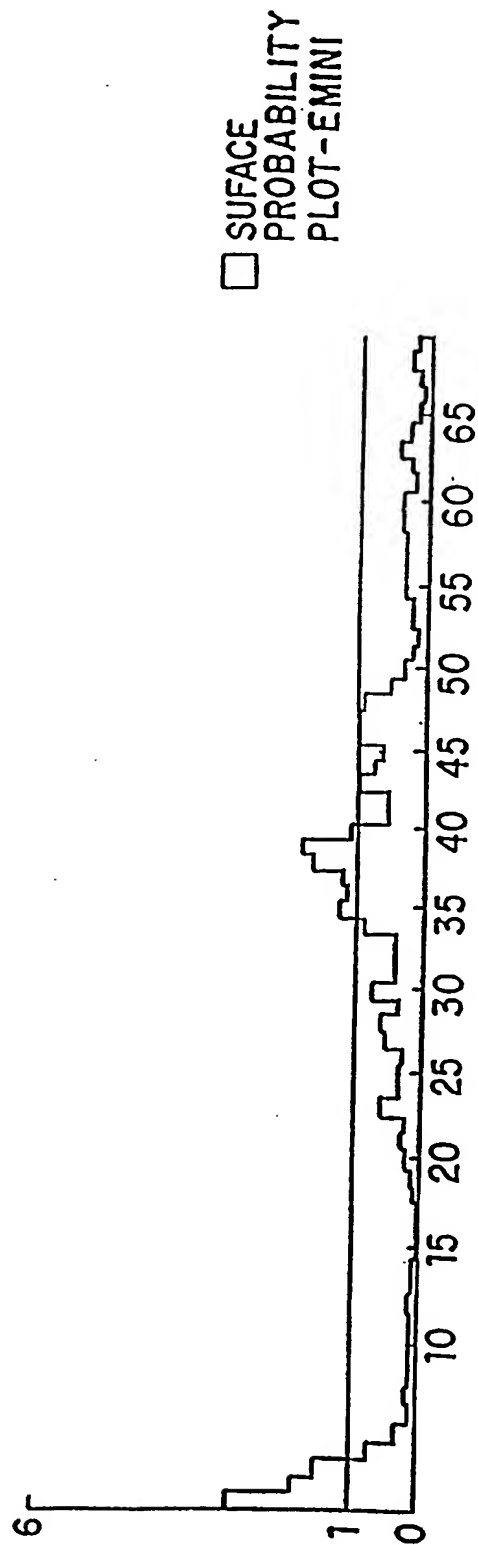
HYDROPHILICITY PLOT-KYTE-DOOLITTLE

FIG. 10D

SURFACE PROBABILITY PLOT-EMINI

FIG.10E

HYDROPHILICITY PLOT-KYTE-DOOLITTLE

FIG.10F

SUFACE PROBABILITY PLOT-EMINI
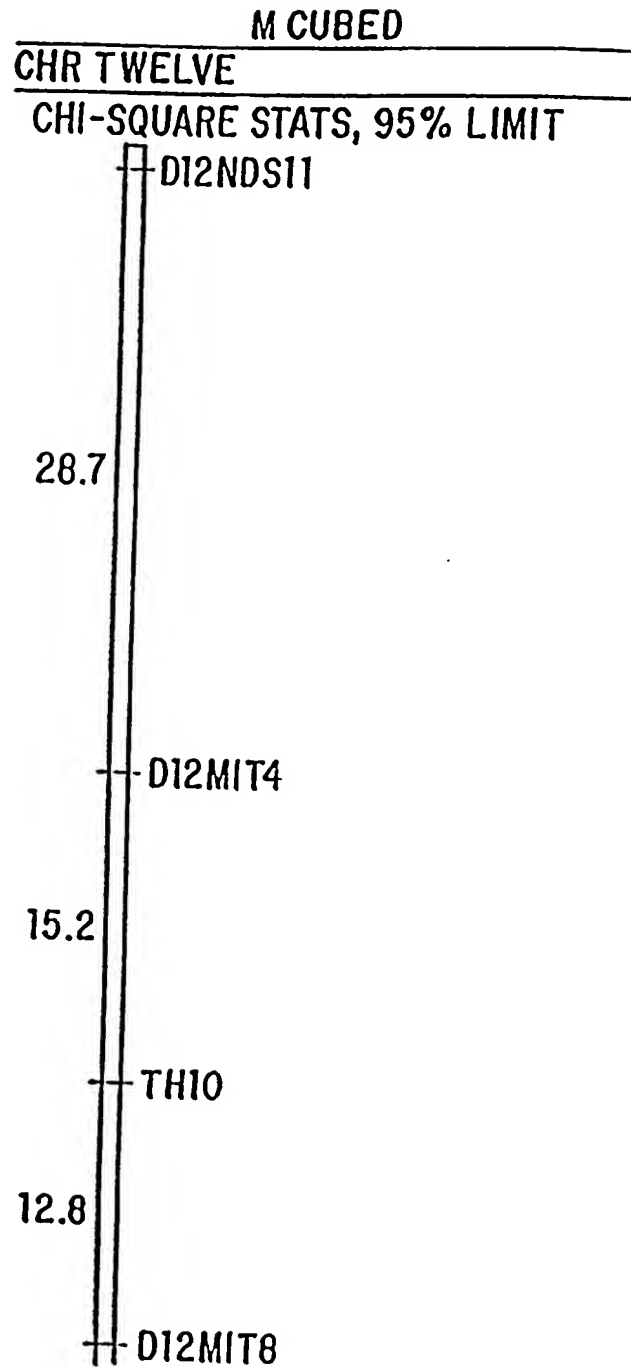
FIG. 11

CGCCAGTGTGCTGGAATTCGGCTTAGAGCATTTCTTTCA
AACCACAGGTTAACACACACTTACTAAAAAGCAATGCTG
TTAGAGGAGAAGGGCTTGGGAGACTCGGCCATTTGAAAC
ANAAGCAAGGCACTCTCCAGGNNCAGCAAGTGGATTCCC
ATTTCCTGCTGAGGGCGGGTTCACACTGAGACTGCACTC
CAGTCAGCGGGAGGAATCACCTGCATTAATGCTTGTCCT
CTGCAGAGCTAGTGTGCCTTCCACTCTGGGTACACTTGG
GTGTCAACATTTCAAAATGATGACCTAAGAGGCTCTCAT
AGTTGGTGATAACTATGGNAGGACAGAAGAACACTGGCT
GTATTGTCTTTTTCTTTCAGCACTAGTGTCTTGGCCCTT
AACTAAAACGGGTTCCATCATCCTCCAAACCAGGAAGAT
AGATTGTTAGACAGGTCCTTTCCCCTCAACT

FIG. 12

TTTNNGGGACAGGGTTTCNCTGTGTATCTCTGGCTGTCC
TGGAACTNACTCTGTAGACCAGGTTGGCCTCGANCTCAG
AAATCTACCTGCCTCTCCCTCCANAGTGCTGGGATTAAN
GGTGTATGCCACCAATNCCCGGCCTTAATATATTNNTAA
ACAACTTCATTTGAATGANATATTGACACTACCCTTGGA
ATAAGAGTNCCCAGAATGANGTACAGGNTTCANGGAATC
ATTTAA

FIG. 14

CTTAGCAGGTGGAGTTGCAGCAGGAAGCCTGGTAGCCAC
ACTCCAATCAGCAGGGGTCCTTGGACTCTCCACATCAAC
AAATGCCATCCTAGGGGCTGCTGGGGCACTGTTGGAGCC
TTGCTCTGAGCTTAGGAGATGACACTTCTATCAGCTCAA
CTCAAAGCCTGTACAGACTACGCAGGAGATGAAGTTCCA
AAAGGCACCTTCAGAACCCTCA

FIG. 15

```
          10        20        30        40        50        60        70
TTTTTTTTT TNGGGAGAGG CTAGCACTGA AATTACAGTT TCAGTGGAAT TTAGAGAAGT AATAACTGCA  70
AAAATTTATT TACACACACA CACACACACA CAGGGCATTT TACCTGTGTA AGTGCAGTTT AATCANCCCC 140
ATTACCTTAT GACCTTGGTT GGCAATGTCT CTAAAGCTTT AAAATTAAAA TAAAATTAAA AAGATGGTTT 210
TCCATCTCAT AAAATCCCCT TTGGGAATGG AAGACTTCCT CTTTGGGGTN TTTTTTAGAG GGAACAGGAG 280
GTAACTGTTA ATTATTTATA CATTCTAATA AACCATGAAT GCACCACATA AAATACTGTA CTCGGGGAGC 350
AAACACTGTN TGGGGGGGTT CTCTCTTACC AGAAGGAACA GGGGGCTTTT CAATGGCTGT GGGC        414
```

FIG. 13

```
remt161g0f   F------------------------------------------------------------
gi/218574/   MRQKAVSLFLCYLLLFTCSGVEAGKKKCSESSDSGSGF-WKALTFMAVGGGLAVAGLP--   CHIMP GENE
gi/32698/g   MRQKAVSVFLCYLLLFTCSGVEAGKKKCSESSDSGSGF-WKALTFMAVGGGLAVAGLP--   HUMAN 6-16
gi/32701/g   ----------------VEAGKKKCSESSDSGSGF-WKALTFMAVGGGLAVAGLP--       HUMAN 6-16
gi/32702/g   --------------------GKKKCSESSDSGSGF-WKALTFMAVGGGLAVAGLP--       HUMAN 6-16
gi/35184/g   MEASAL-------TSSAVTSVAKVVRVASGSAVVLPLARIATVVIGGVVANAAVPMV       HUMAN P27
                                                                          BAND 161

remt161g0f   ---FVFLA------------------------------------------------------
gi/218574/   --ALGFTGAGIAANSVAASLMSMSAILNGGGVPAGGLVATLQSAGVLGLSTSTNAILGAA   BAND 161
gi/32698/g   --ALGFTGAGIAANSVAASLMSMSAILNGGGVPAGGLVATLQSLGAGG----SSVITGNI   CHIMP GENE
gi/32701/g   --ALGFTGAGIAANSVAASLMSMSAILNGGGVPAGGLVATLQSLGAGG---SSVVIGNI    HUMAN 6-16
gi/32702/g   --ALGFTGAGIAANSVAASLMSMSAILNGGGVPAGGLVATLQSLGAGG---SSVVIGNI    HUMAN 6-16
gi/35184/g   LSAMGFTAAGIASSSIAAKMSAAAIANGGGVASGSLVGTLQSLGATGLSGLTKFILGSI    HUMAN 6-16
                                                                          HUMAM P27

remt161g0f   GALLEPCSELRR--------                 BAND 161
gi/218574/   GALMGYATHKYLDSEEDEE   CHIMP GENE
gi/32698/g   GALMRYATHKYLDSEEDEE   HUMAN 6-16
gi/32701/g   GALMRYATHKYLDSEEDEE   HUMAN 6-16
gi/32702/g   GALMRYATHKYLDSEEDEE   HUMAN 6-16
gi/35184/g   GSAIAAVIARFY          HUMAN P27
```

FIG. 16

```
                                        M   F   S   G   L   T   L     6
NGTCGACCCAGCGGTCCGGATTTCCCTCCCAAGTACTC  ATG TTT TCA GGT CTT ACC CTC   60

 N   C   V   L   L   L   Q   L   L   A   R   S   L   E   D   G   Y   K    26
AAC TGT GTC CTG CTG CTG CAA CTA CTT GCA AGG TCA TTG GAA GAT GGT TAT AAG  120

 V   E   V   G   K   N   A   Y   L   P   C   S   Y   T   L   P   T   S   G   T    46
GTT GAG GTT GGT AAA AAT GCC TAT CTG CCC TGC AGT TAC ACT CTA CCT ACA TCT GGG ACA  180

 L   V   P   M   C   W   G   K   G   F   C   P   W   N   S   Q   C   T   N   E   L    66
CTT GTG CCT ATG TGC TGG GGC AAG GGA TTC TGT CCT TGG TCA CAG TGT ACC AAT GAG TTG    240

 L   R   T   D   E   R   N   V   T   Y   Q   L   K   S   S   R   Y   Q   L   K   G    86
CTC AGA ACT GAT GAA AGA AAT GTG ACA TAT CAG AGC AGA TAC CAG CTA AAG GGC    300

 D   L   N   K   G   D   V   S   L   I   I   K   N   V   T   L   D   D   H   G    106
GAT CTC AAC AAA GGA GAT GTG TCT CTG ATC ATA AAG AAT GTG ACT CTG GAT GAC CAT GGG    360

 T   Y   C   C   R   I   Q   F   P   G   L   M   N   D   K   K   L   E   L   K    126
ACC TAC TGC TGC AGG ATA CAG TTC CCT GGT CTT ATG AAT GAT AAA AAA TTA GAA CTG AAA    420

 L   D   I   K   A   K   V   T   P   A   Q   T   A   H   G   D   S   T   T    146
TTA GAC ATC AAA GCA GCC AAG GTC ACT CCA CAG ACT GCC CAT GGG GAC TCT ACT ACA    480

 A   S   P   R   T   L   T   T   E   R   N   G   S   E   T   Q   T   L   V   T    166
GCT TCT CCA AGA ACC CTA ACC ACG GAG AGA AAT GGT TCA GAG ACA CAG ACA CTG GTG ACC    540
```

FIG. 17A

```
 L   H   N   N   G   T   K   I   S   T   W   A   D   E   I   K   D   S   G   186
CTC CAT AAT AAC AAT GGA ACA AAA ATT TCC ACA TGG GCT GAT GAA ATT AAG GAC TCT GGA. 600

 E   T   I   R   T   A   I   H   I   G   V   G   V   S   A   G   L   T   L   A   206
GAA ACG ATC AGA ACT GCT ATC CAC ATT GGA GTG GGA GTC TCT GCT GGG TTG ACC CTG GCA 660

 L   I   I   G   V   L   L   K   W   Y   S   C   K   K   K   L   S   226
CTT ATC ATT GGT GTC TTA ATC CTT AAA TGG TAT TCC TGT AAG AAA AAG TTA TCG AGT 720

 L   S   L   I   T   L   A   N   L   P   P   G   G   L   A   N   A   G   A   V   246
TTG AGC CTT ATT ACA CTG GCC AAC TTG CCT CCA GGA GGG TTG GCA AAT GCA GGA GCA GTC 780

 R   I   R   S   E   E   N   I   Y   T   I   E   E   N   V   Y   E   V   E   N   266
AGG ATT CGC TCT GAG GAA AAT ATC TAC ACC ATC GAG GAG AAC GTA TAT GAA GTG GAG AAT 840

 S   N   E   Y   Y   C   Y   V   N   S   Q   Q   P   S   *   280
TCA AAT GAG TAC TAC TGC TAC GTC AAC AGC CAG CAG CCA TCC TGA CCGCCTCTGGACTGCCACT 903

TTTAAAGGCTCGCCTTCATTTCTGACTTGGTATTTCCCTTKTGGAAAACTATGTGATATGTCACTTGGCAACCTCAT 982

TGGAGGTTCTGACCACAGCCACTGAGAAAAGAGTTCCAGTTTTCTGGGGATAATTAACTCACAAGGGGATTCGACTGTA 1061

ACTCATGCTACACATTGAAATGCTCCATTTTATCCCTGAGTTTCAGGGGATCGGATCTCCCCACTCCAGAGACTTCAATCATG 1140

CGTGTTTGAAGCTCACTCGTGCTTTCATACACATTAGGAATGGTTAGTGTGATGTGTCTTTGAGACATAGAGGTTTGTGGGTATA 1219
```

FIG. 17B

TCCGCAAAGCTCCTGAACAGGTAGGGGGGAATAAAGGGCTAAGATAGGAAGGTGCGGYTCTTTGTTGATGTTGGAAAATC 1298

TTAAAGAAGTTGGTAGCTTTTCT AGAGATTTCTGACCTTGAAAGATTAAGAAAAAGCCAGGTGGCATATGCTTAACAC 1376

GATATAACTTGGGAACCTTAGGCAGGAGGGTGATAAGTTCAAGGTCAGCCAGGGCTATGCTGGTAAGACTGTCTCAMCA 1455

TCCAAAGACGAAAATAAACATAGAGACAGGAGGCTGGAGATGAGGCTCGGACAGTGAGGTGCATTGTGTACAAGCA 1534

CGAGGAATCTATATTTGATCGTAGACCCCACATGAAAAAGCTAGGCCTGGTAGAGCATGCTTGTAGACTCAAGAGATGG 1613

AGAGGTAAAGGCACAACAGATCCCCGGGGCTTGCGTGCAGTCAGCTTAGCCTTAGGTGCTGAGTTCCAAGTCCACAAGAG 1692

TCCCTGTCTCAMAGTAAGATGGRCTGAGTATCTGGCGCATGTCCATGGGGGTTGTCCTCCTCCTCTCAGAAGAGACATGC 1771

ACATGACCCTGCACACACACACACACACACACACACTCCTCGCCTCTGTTAAGACACATGAAATGAAGGTTCTCTCTG 1850

TGCCTGCTACCTCTCTATAACATGTATCTTCACAGGACTCCTCCTCGCCTCTGTTAAGACATGAGTGGGAGCATGGCAG 1929

AGCAGTCCAGTAATTTATTCCAGCACTCAGAAGGCTGGAGCAGAGAAGCGTGGAGAGTTCAGGAGCACTGTGCCCAAACACT 2008

GCCAGACTCTTCTTACACAGAAGAAAGGTTACCCGCAAGCAGCCTGCTGTCTGTAAAGGAAACCCTGCGAAAGGCAAA 2087

CTTTGACTGTTGTGTGCTCAAGGGGGAACTGACTCAGACACAACTTCTCCATTCCTGGAGGAAACTGGAGCTGTTTCTGACA 2166

GAAGAACAACCGGTGACTGGGACATACGAGAGGCAGAGCTCTTGCAGCAATCTATATAGTCAGCAAAATATTCTTTGGGA 2245

FIG. 17C

GGACAGTCGTCACCAAATTGATTTCCAAGCCGGTGGACCTCAGTTTCATCTGGCTTACAGCTGCCTGCCCAGTGCCCTT 2324

GATCTGTTGCTGGCTCCCATCTATAACAGAATCAAATTAAATAGACCCCGAGTGAAAATATTAAGTGAGCAGAAAGGTAG 2403

CTTTGTTCAAAGATTTTTTGCATTGGGGAGCAACTGTGTACATCAGAGGACATCTGTTAGTGAGGACACCAAAACCTG 2482

TGGTACCGTTTTTCATGTATGAATTTGTTGTTTAGGTTGCTTCTAGCTAGCTGTGGGAGGTCCTGGCTTTCTTAGGTG 2561

GGTATGGAAGGAGACCATCTAACAAAATCCATTAGAGATAACAGCTCTCATGCAGAGGGAAAACTAATCTCAAATGT 2640

TTTAAAGTAATAAAACTGTACTGGCAAAGTACTTTGAGCATAAAAAAAAAAAAAAAAGGGGCGGCCGC 2710

FIG. 17D

FIG. 18

FIG. 19

FIG. 20

FIG. 21

```
                  M   T   L   T   A   H   L   S   Y   F   L   V   L    13
C CGGGTCGACC CACGCGTCCG ATG ACA CTG ACT GCC CAC CTC TCC TAC TTT CTG GTC CTG   60

  L   A   G   Q   G   L   S   D   S   L   L   T   K   D   A   G   P   R   P    33
TTG GCG GGC CAA GGC CTC AGT GAC TCC CTC ACC AAG GAT GCA GGT CCC CGC CCA        120

  L   E   K   E   V   F   K   L   F   Q   I   R   F   N   R   S   Y   W   N    53
CTG GAG AAG GAA GTC TTC AAG CTG TTC CAG ATC CGG TTC AAC CGG AGT TAC TGG AAC    180

  P   A   E   Y   T   R   R   L   S   I   F   A   H   N   L   A   Q   A   Q   R    73
CCA GCA GAG TAC ACT CGC CGT CTG AGC ATC TTT GCC CAC AAT CTG GCT CAG GCT CAA AGG 240

  L   Q   Q   E   D   L   G   T   A   E   F   G   E   T   P   F   S   D   L   T    93
CTA CAG CAA GAA GAC TTG GGT ACA GCT GAG TTT GGA GAG ACT CCA TTC AGT GAC CTC ACA 300

  E   E   F   G   Q   L   Y   G   Q   E   R   S   P   E   R   T   P   N   M    113
GAG GAG TTT GGC CAG TTA TAC GGG CAG GAG AGG TCA CCA GAA AGG ACC CCC AAC ATG     360

  T   K   K   V   E   S   N   T   W   G   E   S   V   P   R   T   C   D   W   R    133
ACC AAA AAG GTA GAG TCT AAC ACG TGG GGG GAA TCT GTG CCC CGC ACC TGT GAC TGG CGT  420

  K   A   K   N   I   I   S   S   V   K   N   Q   G   S   C   K   C   W   A    153
AAA GCA AAG AAC ATC ATC TCG TCG GTC AAG AAC CAG GGA AGC TGC AAA TGC TGG GCC      480

  M   A   A   D   N   I   Q   A   L   W   R   I   K   H   Q   Q   F   V   D    173
ATG GCA GCT GAC AAC ATC CAG GCT CTG TGG CGC ATC AAA CAC CAG CAG TTT GTG GAC      540
```

FIG. 22A

```
      V   S   V   Q   E   L   L   D   C   E   R   C   G   N   G   G   F              193
     GTC TCT GTG CAG GAG CTG CTG GAC TGC GAA CGC TGT GGA AAT GGT GGC TTC             600

      V   W   D   A   Y   L   T   V   L   N   N   S   G   L   A   S   E   K   D   Y   213
     GTG TGG GAC GCA TAT CTA ACT GTC CTC AAC AAC AGT GGC CTG GCC AGT GAA AAG GAT TAT 660

      P   F   Q   G   D   R   K   P   H   R   C   L   A   K   K   Y   K   K   V   A   233
     CCA TTC CAG GGG GAC AGA AAG CCT CAC AGA TGC CTA GCC AAG AAG TAC AAG AAG GTG GCC 720

      W   I   Q   D   F   T   M   L   S   N   N   E   Q   A   I   A   H   Y   L   A   253
     TGG ATC CAG GAT TTC ACC ATG TTG TCC AAT AAT GAG CAG GCA ATT GCC CAC TAC CTG GCC 780

      V   H   G   P   I   T   V   T   I   N   M   K   L   Q   H   Y   Q   K   G       273
     GTG CAT GGA CCT ATC ACC GTG ACC ATC AAC ATG AAA CTA CTC CAG CAT TAC CAG AAG GGT 840

      V   I   K   A   T   P   S   S   C   D   P   R   Q   V   D   H   S   V   L   L   293
     GTC ATC AAG GCT ACA CCC AGC TCC TGT GAC CCT CGG CAA GTG GAC CAC TCT GTC TTG CTG 900

      V   G   F   G   K   E   K   E   G   M   Q   T   G   T   V   L   S   H   S   R   313
     GTG GGC TTT GGC AAG GAG AAA GAG GGC ATG CAG ACA GGG ACA GTC TTG TCC CAT TCT CGA 960

      K   R   H   S   S   P   Y   W   I   L   K   N   S   W   G   A   H   W   G       333
     AAA CGT CAC TCC TCC CCA TAC TGG ATC CTG AAG AAC TCC TGG GGA GCT CAC TGG GGC     1020

      E   K   G   Y   F   R   L   Y   R   G   N   N   T   C   G   V   T   K   Y   P   353
     GAG AAG GGT TAC TTC AGG CTG TAT CGG GGA AAC AAC ACC TGT GGA GTC ACC AAG TAT CCC 1080
```

FIG. 22B

```
 F    T    A    Q    V    D    S    P    V    K    K    A    R    T    S    C    P    P    *     371
TTC  ACA  GCT  CAA  GTG  GAC  TCA  CCA  GTA  AAG  AAG  GCA  CGG  ACC  TCT  TGT  CCT  CCC  TGA  AGG  1140

CAGCAGVCAC  TCTTCTGCTT  CTCCCACATG  GCCACTGCCC  CTTGTCAGCC  CTGCCCACAT  CCTCTCTGTA              1210

TGGCTTCATA  AACCAAGACT  GCTCCGTGAA  AAAAAAAAAAAAAA                                             1257
```

FIG. 22C

```
                    M   T   L   T   A   H   L   S   Y   F   L   V   L        13
C CGGGTCGACC CACGGGTCCG ATG ACA CTG ACT GCC CAC CTC TCC TAC TTT CTG GTC CTG  60

L   A   G   Q   G   L   S   D   S   L   L   T   K   D   A   G   P   R   P     33
TTG TTA GCG GGC CAA GGC CTC AGT GAC TCC CTC CTC ACC AAG GAT GCA GGT CCC CGC CCA  120

L   E   L   K   E   V   F   K   L   F   Q   I   R   F   N   R   S   Y   W   N  53
CTG GAG CTG AAG GAA GTC TTC AAG CTG TTC CAG ATC CGG TTC AAC CGG AGT TAC TGG AAC  180

P   A   E   Y   T   R   R   L   S   I   F   A   H   N   L   A   Q   Q   A   R  73
CCA GCA GAG TAC ACT CGC CGT CTG AGC ATC TTT GCC CAC AAT CTG GCT CAG CAA GCT CAA AGG  240

L   Q   E   D   L   G   T   A   E   F   G   E   T   P   F   S   D   L   T      93
CTA CAG CAA GAA GAC TTG GGT ACA GCT GAG TTT GGA GAG ACT CCA TTC AGT GAC CTC ACA  300

E   E   F   G   Q   L   Y   G   Q   E   R   S   P   E   R   T   P   N   M     113
GAG GAG TTT GGC CAG TTA TAC GGG CAG GAG AGG TCA CCA GAA AGG ACC CCC AAC ATG  360

T   K   K   V   E   S   N   T   W   G   E   S   V   P   R   T   C   D   W   R  133
ACC AAA AAG GTA GAG TCT AAC ACG TGG GGG GAA TCT GTG CCC CGC ACC TGT GAC TGG CGT  420

K   A   K   N   I   I   S   S   V   K   N   Q   G   S   C   K   C   W   A     153
AAA GCA AAG AAC ATC ATC TCG TCG GTC AAG AAC CAG GGA AGC TGC AAA TGC TGG GCC  480

M   A   A   D   N   I   Q   A   L   W   R   I   K   H   Q   Q   F   V   D     173
ATG GCA GCT GCC GAC AAC ATC CAG GCT CTG TGG CGC ATC AAA CAC CAG CAG TTT GTG GAC  540
```

Pre-Pro

FIG.23A

```
        V   S   V   Q   E   L   L   D   C   E   R   C   G   N   G   C   N   G   G   F
       GTC TCT GTG CAG GAG CTG CTG GAC TGC GAA CGC GGA AAT GGT GGC TGC AAT GGT GGC TTC    193
                                                                                          600

        V   W   D   A   Y   L   T   Y   L   N   N   S   G   L   A   S   E   K   D   Y
       GTG TGG GAC GCA TAT CTA ACT GTC CTC AAC AGT GGC CTG GCC AGT GAA AAG GAT TAT        213
                                                                                          660

        P   F   Q   G   D   R   K   P   H   R   C   L   A   K   K   Y   K   K   V   A
       CCA TTC CAG GGG GAC AGA AAG CCT CAC AGA TGC CTA GCC AAG AAG TAC AAG AAG GTG GCC    233
                                                                                          720

        W   I   Q   D   F   T   M   L   S   N   N   E   Q   A   I   A   H   Y   L   A
       TGG ATC CAG GAT TTC ACC ATG TTG TCC AAT AAT GAG CAG GCA ATT GCC CAC TAC CTG GCC    253
                                                                                          780

        V   H   G   P   I   T   V   T   I   N   M   K   L   L   Q   H   Y   Q   K   G
       GTG CAT GGA CCT ATC ACC GTG ACC ATC AAC ATG AAA CTA CTC CAG CAT TAC CAG AAG GGT    273
                                                                                          840

        V   I   K   A   T   P   S   S   C   D   P   R   Q   V   D  [H]  S   V   L   L
       GTC ATC AAG GCT ACA CCC AGC AGC TGT GAC CCT CGG CAA GTG GAC CAC TCT GTC TTG CTG    293
                                                                                          900

        V   G   F   G   K   E   K   E   G   M   Q   T   G   T   V   L   S   H   S   R
       GTG GGC TTT GGC AAG GAG AAA GAG GGC ATG CAG ACA GGG ACA GTC TTG TCC CAT TCT CGA    313
                                                                                          960

        K   R   H   S   S   P   Y   W   I   L   K  [N] [W]  G   A   H   W   G
       AAA CGT CGC CAC TCC TCC CCA TAC TGG ATC CTG AAG AAC TCC TGG GGA GCT CAC TGG GGC    333
                                                                                         1020
```

MATURE

FIG.23B

```
 E   K   G   Y   F   R   L   Y   R   G   N   N   T   C   G   V   T   K   Y   P   353
GAG AAG GGT TAC TTC AGG CTG TAT CGG GGA AAC AAC ACC TGT GGA GTC ACC AAG TAT CCC 1080

 F   T   A   Q   V   D   S   P   V   K   K   A   R   T   S   C   P   P   *         371
TTC ACA GCT CAA GTG GAC TCA CCA GTA AAG AAG GCA CGG ACC TCT TGT CCT CCC TGA AGG  1140

CAGCAGVCAC TCTTCTGCTT CTCCCACATG GCCACTGCCC CTTGTCAGCC CTGCCCACAT CCTCTCTGTA       1210

TGGCTTCATA AACCAAGACT GCTCCGTGAA AAAAAAAAAA AAAAAAAAA                              1257
```

FIG.23C

```
                                              M  F  S  H  L  P      6
       CGCTAACAGAGGTGTCCTCTGACTTTTCTTCTGCAAGCTCC ATG TTT TCA CAT CTT CCC   18

 F  D  C  V  L  L  L  L  L  L  L  T  R  S  S  E  V  E  Y    26
TTT GAC TGT GTC CTG CTG CTG CTG CTG CTA CTA CTT ACA AGG TCC TCA GAA GTG GAA TAC   78

 R  A  E  V  G  Q  N  A  Y  L  P  C  F  Y  T  P  A  A  P  G    46
AGA GCG GAG GTC GGT CAG AAT GCC TAT CTG CCC TGC TTC TAC ACC CCA GCC GCC CCA GGG  138

 N  L  V  P  V  C  W  G  K  G  A  C  P  V  F  E  C  G  N  V    66
AAC CTC GTG CCC GTC TGC TGG GGC AAA GGA GCC TGT CCT GTG TTT GAA TGT GGC AAC GTG  198

 V  L  R  T  D  E  R  D  V  N  Y  W  T  S  R  Y  W  L  N  G    86
GTG CTC AGG ACT GAT GAA AGG GAT GTG AAT TAT TGG ACA TCC AGA TAC TGG CTA AAT GGG  258

 D  F  R  K  G  D  V  S  L  T  I  E  N  V  T  L  A  D  S  G   106
GAT TTC CGC AAA GGA GAT GTG TCC CTG ACC ATA GAG AAT GTG ACT CTA GCA GAC AGT GGG  318

 I  Y  C  C  R  I  Q  I  P  G  I  M  N  D  E  K  F  N  L  K   126
ATC TAC TGC TGC CGG ATC CAA ATC CCA GGC ATA ATG AAT GAT GAA AAA TTT AAC CTG AAG  378

 L  V  I  K  P  A  K  V  T  P  A  P  T  L  Q  R  D  F  T  A   146
TTG GTC ATC AAA CCA GCC AAG GTC ACC CCT GCA CCG ACT CTG CAG AGA GAC TTC ACT GCA  438

 A  F  P  R  M  L  T  T  R  G  H  G  P  A  E  T  Q  T  L  G   166
GCC TTT CCA AGG ATG CTT ACC ACC AGG GGA CAT GGC CCA GCA GAG ACA CAG ACA CTG GGG  498

 S  L  P  D  I  N  L  T  Q  I  S  T  L  A  N  E  L  R  D  S   186
AGC CTC CCT GAT ATA AAT CTA ACA CAA ATA TCC ACA TTG GCC AAT GAG TTA CGG GAC TCT  558

 R  L  A  N  D  L  R  D  S  G  A  T  I  R  I  G  I  Y  I  G   206
AGA TTG GCC AAT GAC TTA CGG GAC TCT GGA GCA ACC ATC AGA ATA GGC ATC TAC ATC GGA  618

 A  G  I  C  A  G  L  A  L  A  L  I  F  G  A  L  I  F  K  W   226
GCA GGG ATC TGT GCT GGG CTG GCT CTG GCT CTT ATC TTC GGC GCT TTA ATT TTC AAA TGG  678

 Y  S  H  S  K  E  K  I  Q  N  L  S  L  I  S  L  A  N  L  P   246
TAT TCT CAT AGC AAA GAG AAG ATA CAG AAT TTA AGC CTC ATC TCT TTG GCC AAC CTC CCT  738

 P  S  G  L  A  N  A  V  A  E  G  I  R  S  E  E  N  I  Y  T   266
CCC TCA GGA TTG GCA AAT GCA GTA GCA GAG GGA ATT CGC TCA GAA GAA AAC ATC TAT ACC  798

 I  E  E  N  V  Y  E  V  E  E  P  N  E  Y  Y  C  Y  V  S  S   286
ATT GAA GAG AAC GTA TAT GAA GTG GAG GAG CCC AAT GAG TAT TAT TGC TAT GTC AGC AGC  858

 R  Q  Q  P  S  Q  P  L  G  C  R  F  A  M  P           301
AGG CAG CAA CCC TCA CAA CCT TTG GGT TGT CGC TTT GCA ATG CCA TAGATCCAACCACCTTATT  903

TTTGAGCTTGGTGTTTTGTCTTTTTCAGAAACTATGAGCTGTGTCACCTGACTGGTTTTGGAGGTTCTGTCCACTGCTA

TGGAGCAGAGTTTTCCCATTTTCAGAAGATAATGACTCACATGGGAATTGAACTGGGACCTGCACTGAACTTAAACAGG

CATGTCATTGCCTCTGTATTTAAGCCAACAGAGTTACCCAACCCAGAGACTGTTAATCATGGATGTTAGAGCTCAAACG

GGCTTTTATATACACTAGGAATTCTTGACGTGGGGTCTCTGGAGCTCCAGGAAATTCGGG CACATCATATGTCCATGA

AACTTCAGATAAACTAGGRAAAACTGGGTGCTGAGGTGAAAGCATAACTTTTTTGGCACAGAAAGTCTAAAGGGGCCAC

TGATTTTCAAAGAGATCTGTGATCCCTTTTTGTTTTTTGTTTTTGAGATGGAGTCTTGCTCTGTTGCCCAGGCTGGAGT

GCAATGGCACAATCTCGGCTCACTGCAAGCTCCGCCTCCTGGGTTCAAGCGATTCTCCTGCCTCAGCCTCCTGAGTGGC

TGGGATTACAGGCATGCACCACCATGCCCAGCTAATTTGTTGTATTTTTAGTAGAGACAGGGTTTCACCATGTTGGCCA
```

FIG. 24

GTGTGGTCTCAAACTCCTGACCTCATGATTTGCCTGCCTCGGCCTCCCAAAGCACTGGGATTACAGGCGTGAGCCACCA

CATCCAGCCAGTGATCCTTAAAAGATTAAGAGATGACTGACTAGGTCTACCTTGATCTTGAAGATTCCCTTGGAATGT

TGAGATTTAGGCTTATTTGAGCACTACCTGCCCAACTGTCAGTGCCAGTGCATAGCCCTTCTTTTGTCTCCCTTATGAA

GACTGCCCTGCAGGGCTGAGATGTGGCAGGAGCTCCCAGGGAAAAAGGAAGTGCATTTGATTGGTGTGTATTGGCCAAG

TTTTGCTTGTTGTGTGCTTGAAAGAAAATATCTCTGACCAACTTCTGTATTCGTGGACCAAACTGAAGCTATATTTTTC

ACAGAAGAAGAAGCAGTGACGGGGACACAAATTCTGTTGCCTGGTGGAAAGAAGGCAAAGGCCTTCAGCAATCTATATT

ACCAGCGCTGGATCCTTTGACAGAGAGTGGTCCCTAAACTTAAATTTCAAGACGGTATAGGCTTGATCTGTCTTGCTTA

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AAAAAAAAAA

FIG. 24 (cont'd.)
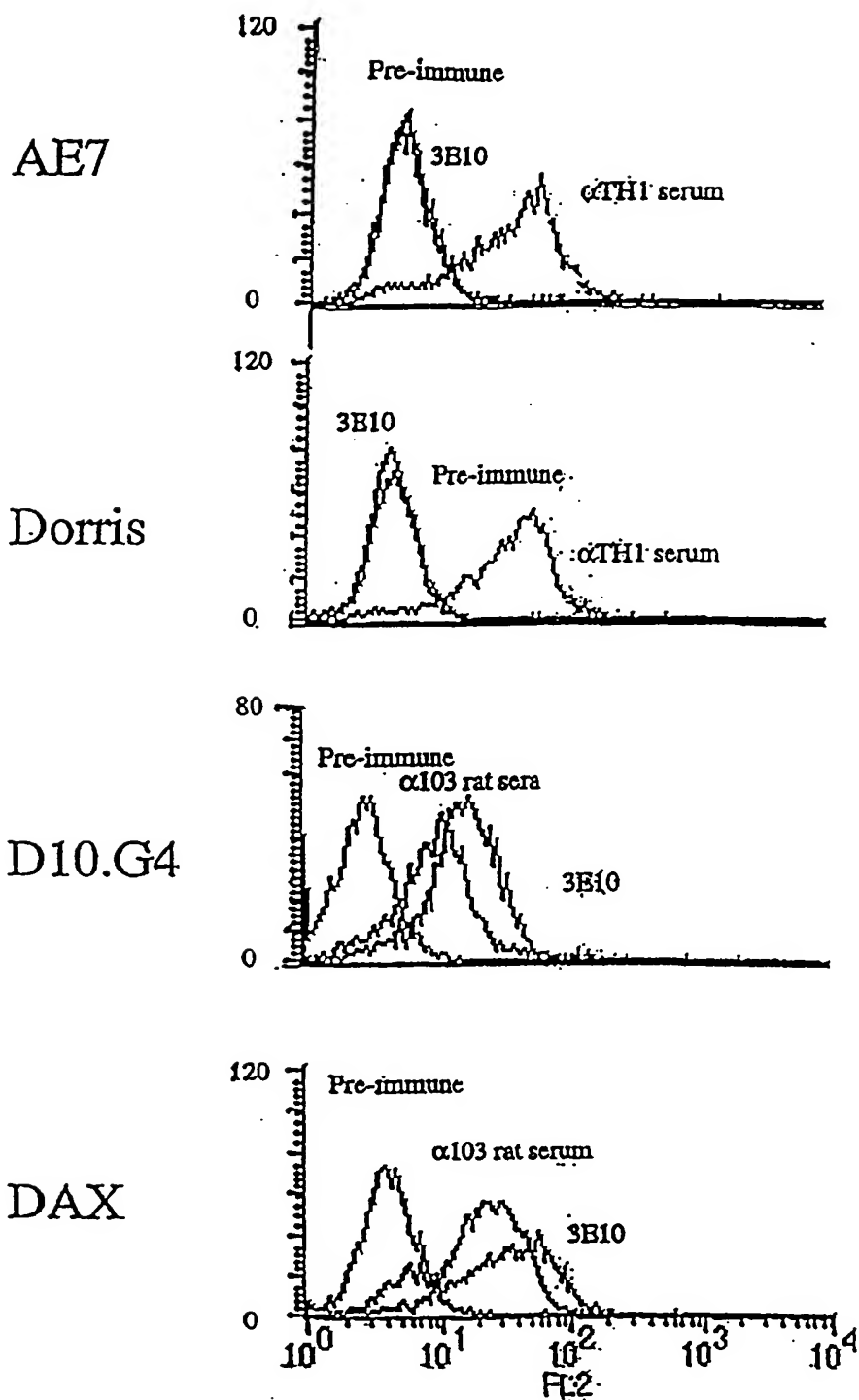
FIGURE 25
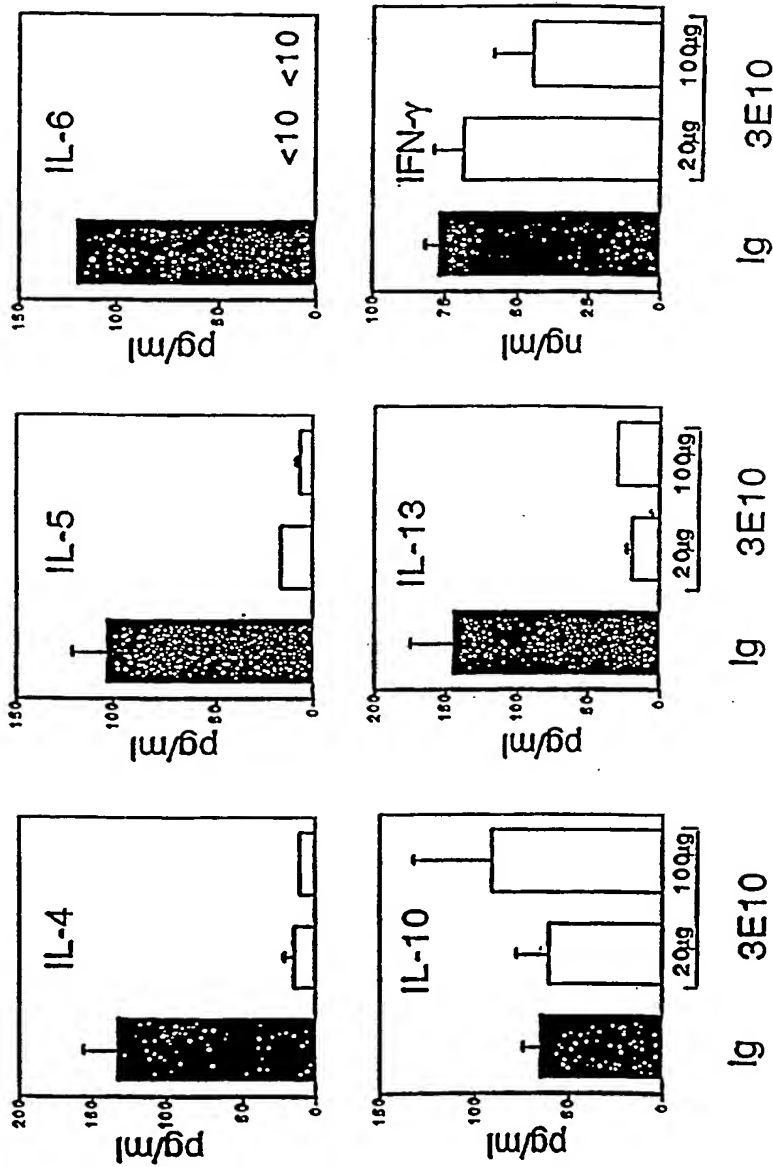
FIGURE 26
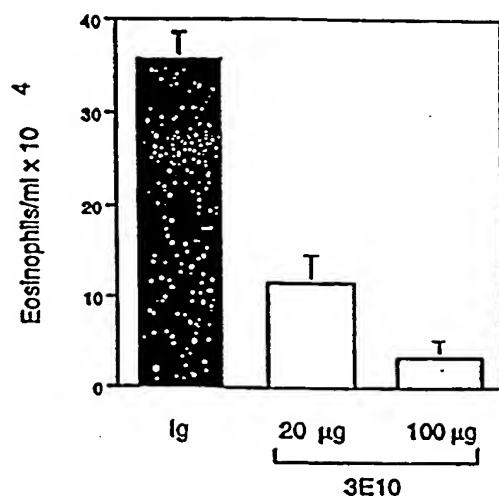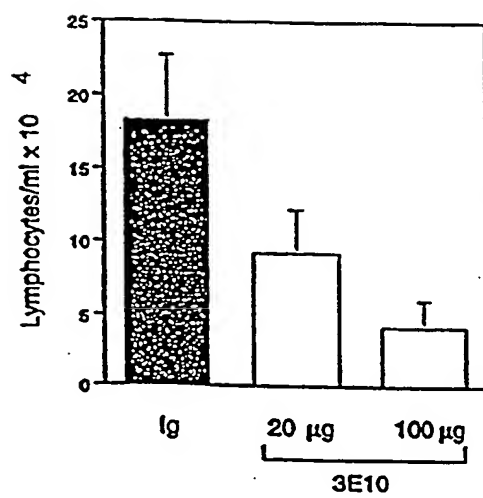
FIGURE 27 A

FIGURE 27 B
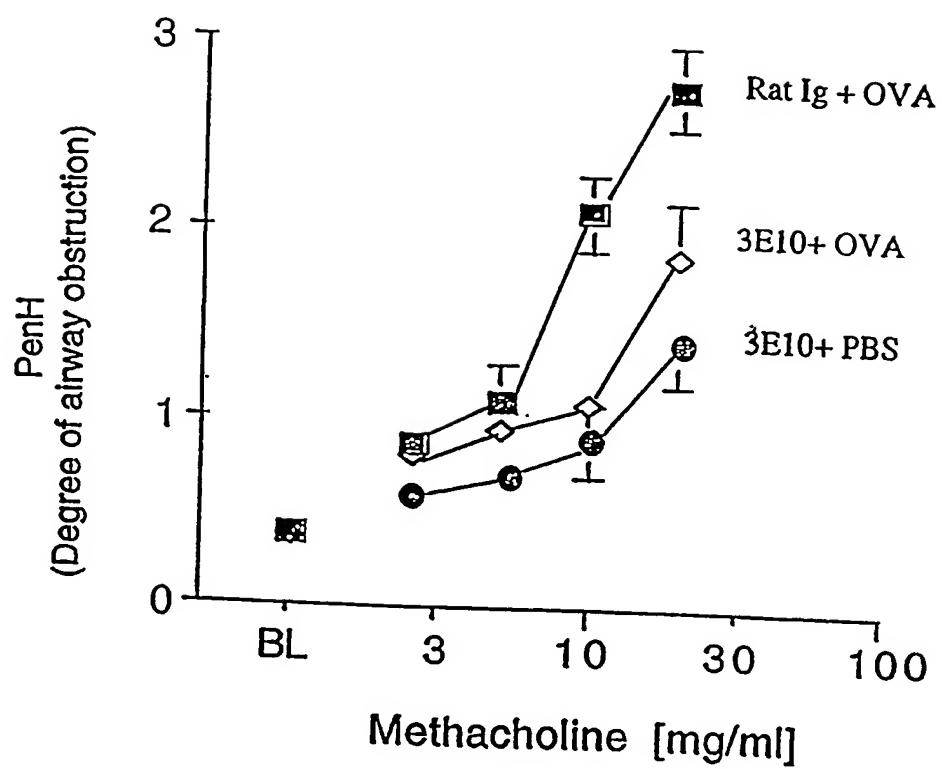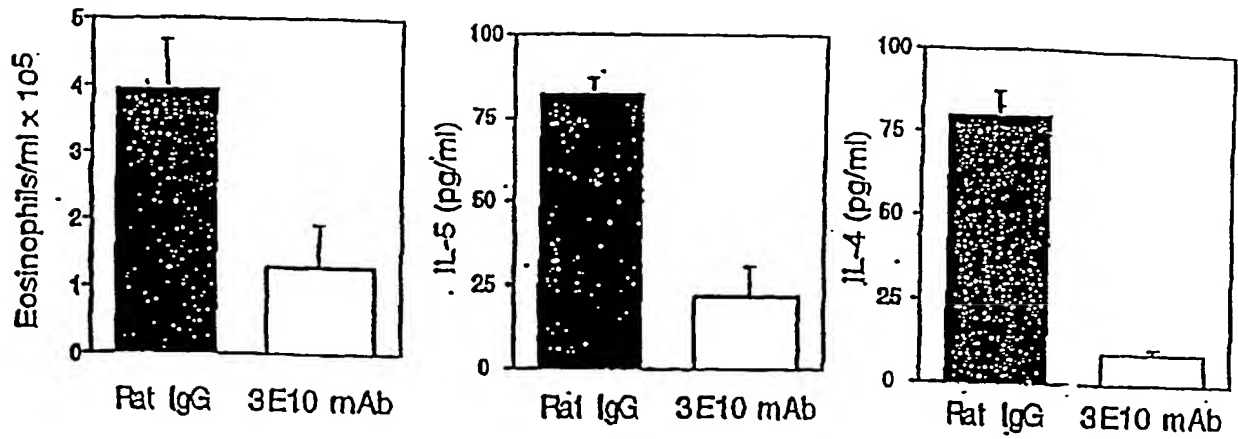
FIGURE 28
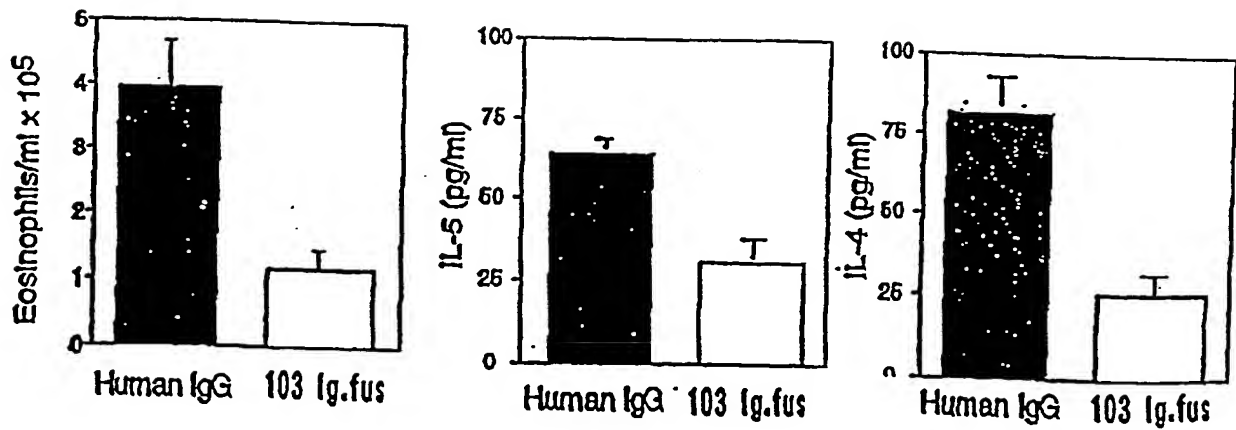
FIGURE 29 A

FIGURE 29 B
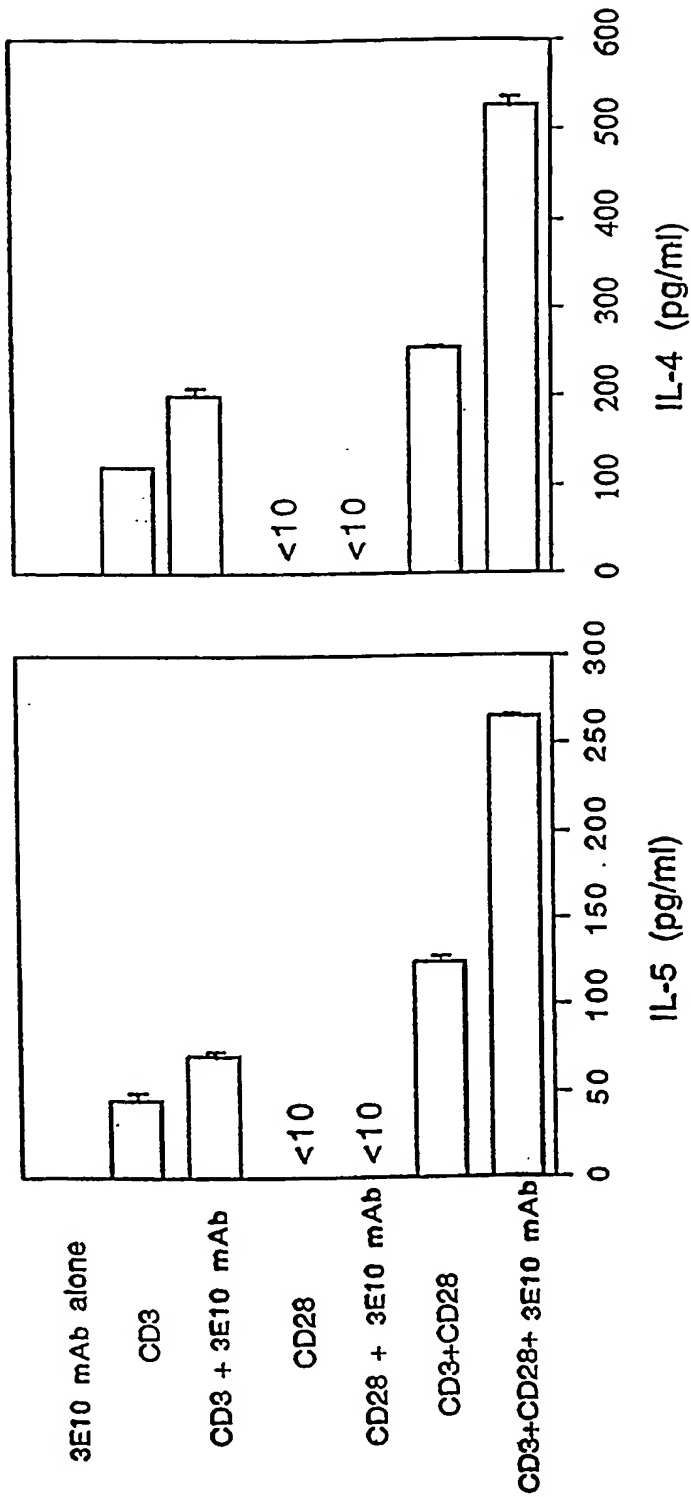
FIG. 30

Renal histology at 72hrs post reperfusion

+a200

+RbIg
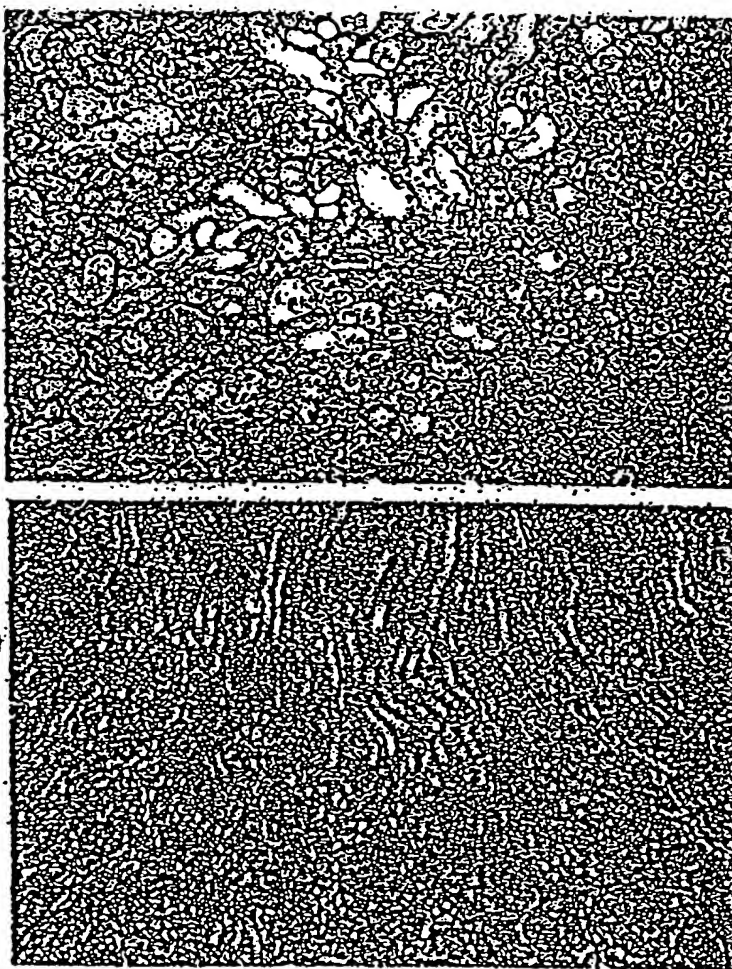
FIG. 31

Blockage of gene 200 during renal ischemia/reperfusion injury
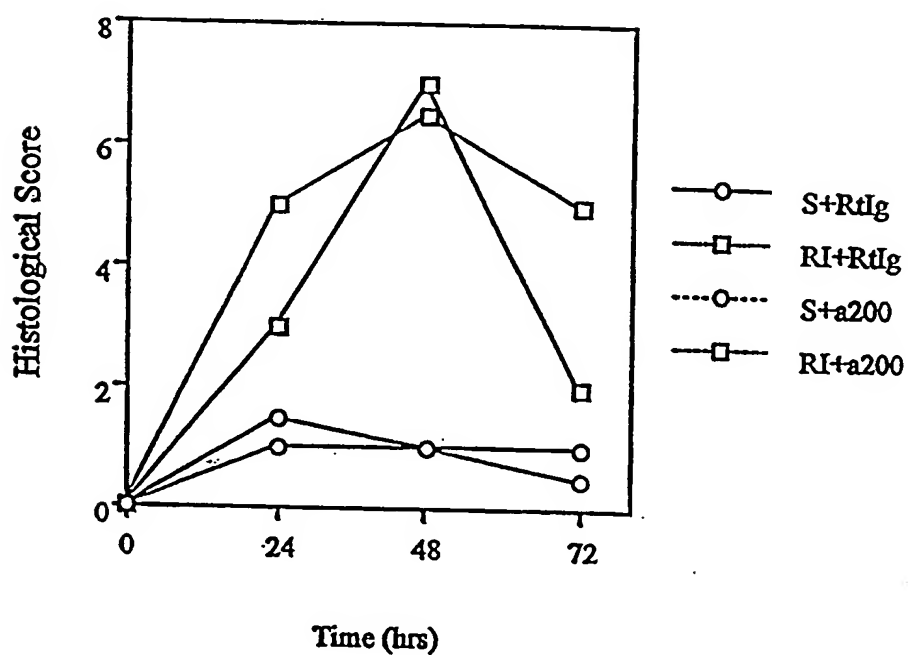
FIG. 32

SEQUENCE LISTING

<110> Millennium Pharmaceuticals, Inc.

<120> COMPOSITIONS AND METHODS FOR THE TREATMENT AND
      DIAGNOSIS OF IMMUNE DISORDERS

<130> 7853-125-228

<140>
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<150> 09/324,986
<151> 1999-06-02

<160> 49

<170> PatentIn Ver. 2.0

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tagcaggatg ggggagattt ggcaataagt cagctccctc tgaaactctg agagaagtca 180

acatcactgt catagacaga aaaatctgca atgatgaaaa acactataat tttcatcctg 240

taattggtct aaacatgatt tgggcagggg acctccccgg cggaaaggac tcctgcaatg 300

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gggcagtaaa tcgtcctggg gtctggaaaa tgaggcttta attgtgagat gcccccaaag 180

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tttgcaccga ccaatctcca atctcctgta agattcaaaa gggcaagcaa gaggcggtga 180

ccgttcacga aagctaaaat cccatgctat tgaacatgaa gacttctgat gcttaaatct 240

cattaactgc tttaagtcac tcccaggagc ttggatccca acttctagca gtaatagtct 300

gtgtaaaaaa aaaaaaaaaa tcagtctaca accactctct aaatgcatgg atgaactcat 360

cagaacatca aaacccaagg aaaccctaag agagaagaat tctaataaaa agaattttac 420

attgaaaact tacaaggcaa ggtccctttc cctgctgaca gcctaagaag tgatgtaact 480

gccactgtga agaccatggc gatgaacagc atgtgcattg aagagcagcg ccacctcgaa 540

cactatttgt tcccggtggt ctacataatt gtgtttatag tcagcgtccc agccaacatc 600

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actttcgtcc tatgctttac ccccttccac gtgatggtgc tcatccgctg cgttttagag 1260

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acggggagag ctgatatgtg aacatatta aaattgtgta ctaggaaaca caatagacac 1440

caagggaaaa aaagggacat actttctgtg tccacaagag atgctgtaga attagagatt 1500

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tggaaaagaa atctggcata gtagaaccca gtggaaatag tttgaaggta cattgtatga 1620

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ctcctatgtt ggctttatta agtaaggtat agaaatgtat tatcttgtat gtattctaat 1680

gactaggcat cattgtttta gtaccaattc tctttgcctc tatgttataa cccctaagaa 1740

gcacgcggga ctgttcgtct ttaaatcagt ggccattcta tctgactact atgacttttt 1800

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aaaaaaaaaa agggc                                                 2055
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agcaaggcac tctccaggnn cagcaagtgg attcccattt cctgctgagg gcgggttcac 180

actgagactg cactccagtc agcgggagga atcacctgca ttaatgcttg tcctctgcag 240

agctagtgtg ccttccactc tgggtacact tgggtgtcaa catttcaaaa tgatgaccta 300

agaggctctc atagttggtg ataactatgg naggacagaa gaacactggc tgtattgtct 360

ttttctttca gcactagtgt cttggccctt aactaaaacg ggttccatca tcctccaaac 420

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```

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3

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agtgcagttt aatcancccc attaccttat gaccttggtt ggcaatgtct ctaaagcttt 180

aaaattaaaa taaaattaaa aagatggttt tccatctcat aaaatcccct ttgggaatgg 240

aagacttcct ctttgggggtn ttttttagag ggaacaggag gtaactgtta attatttata 300

cattctaata aaccatgaat gcaccacata aaatactgta ctcggggagc aaacactgtn 360

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gtatgccacc aatncccggc cttaatatat tnntaaacaa cttcatttga atganatatt 180

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ctctgagctt aggagatgac acttctatca gctcaactca aagcctgtac agactacgca 180

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```

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4

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gttgaggttg gtaaaaatgc ctatctgccc tgcagttaca ctctacctac atctgggaca  180

cttgtgccta tgtgctgggg caagggattc tgtccttggt cacagtgtac caatgagttg  240

ctcagaactg atgaaagaaa tgtgacatat cagaaatcca gcagatacca gctaaagggc  300

gatctcaaca aaggagatgt gtctctgatc ataaagaatg tgactctgga tgaccatggg  360

acctactgct gcaggataca gttccctggt cttatgaatg ataaaaaatt agaactgaaa  420

ttagacatca aagcagccaa ggtcactcca gctcagactg cccatgggga ctctactaca  480

gcttctccaa gaaccctaac cacggagaga aatggttcag agacacagac actggtgacc  540

ctccataata caatggaac aaaaatttcc acatgggctg atgaaattaa ggactctgga  600

gaaacgatca gaactgctat ccacattgga gtgggagtct ctgctgggtt gaccctggca  660

cttatcattg gtgtcttaat ccttaaatgg tattcctgta agaaaaagaa gttatcgagt  720

ttgagcctta ttacactggc caacttgcct ccaggagggt tggcaaatgc aggagcagtc  780

aggattcgct ctgaggaaaa tatctacacc atcgaggaga cgtatatga agtggagaat  840

tcaaatgagt actactgcta cgtcaacagc cagcagccat cctgaccgcc tctggactgc  900

cacttttaaa ggctcgcctt catttctgac tttggtattt ccctttktgg aaaactatgt  960

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tgctccattt tatccctgag tttcagggat cggatctccc actccagaga cttcaatcat 1140

gcgtgttgaa gctcactcgt gctttcatac attaggaatg gttagtgtga tgtctttgag 1200

acatagaggt ttgtggtata tccgcaaagc tcctgaacag gtaggggggaa taaagggcta 1260

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aggctcggac agtgaggtgc attgtgtaca agcacgagga atctatattt gatcgtagac 1560

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aaggcacaac agatccccgg ggcttgcgtg cagtcagctt agcctaggtg ctgagttcca 1680

agtccacaag agtccctgtc tcamagtaag atggrctgag tatctggcgc atgtccatgg 1740
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5

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cacacacaca cacacacaca cacacacaca tgawatgaag gttctctctg tgcctgctac 1860

ctctctataa catgtatctc tacaggactc tcctctgcct ctgttaagac atgagtggga 1920

gcatggcaga gcagtccagt aatttattcc agcactcaga aggctggagc agaagcgtgg 1980

agagttcagg agcactgtgc ccaacactgc cagactcttc ttacacaaga aaaaggttac 2040

ccgcaagcag cctgctgtct gtaaaaggaa accctgcgaa aggcaaactt tgactgttgt 2100

gtgctcaagg ggaactgact cagacaactt ctccattcct ggaggaaact ggagctgttt 2160

ctgacagaag aacaaccggt gactgggaca tacgaaggca gagctcttgc agcaatctat 2220

atagtcagca aaatattctt tgggaggaca gtcgtcacca aattgatttc caagccggtg 2280

gacctcagtt tcatctggct tacagctgcc tgcccagtgc ccttgatctg tgctggctcc 2340

catctataac agaatcaaat taaatagacc ccgagtgaaa atattaagtg agcagaaagg 2400

tagctttgtt caaagatttt tttgcattgg ggagcaactg tgtacatcag aggacatctg 2460

ttagtgagga caccaaaacc tgtggtaccg ttttttcatg tatgaatttt gttgtttagg 2520

ttgcttctag ctagctgtgg aggtcctggc tttcttaggt gggtatggaa gggagaccat 2580

ctaacaaaat ccattagaga taacagctct catgcagaag ggaaaactaa tctcaaatgt 2640

tttaaagtaa taaaactgta ctggcaaagt actttgagca taaaaaaaaa aaaaaaaaaa 2700

gggcggccgc                                                         2710
```

```
<210> 9
<211> 337
<212> PRT
<213> Mus musculus

<400> 9
Met Ala Met Asn Ser Met Cys Ile Glu Glu Gln Arg His Leu Glu His
1               5                   10                  15

Tyr Leu Phe Pro Val Val Tyr Ile Ile Val Phe Ile Val Ser Val Pro
            20                  25                  30

Ala Asn Ile Gly Ser Leu Cys Val Ser Phe Leu Gln Ala Lys Lys Glu
        35                  40                  45

Asn Glu Leu Gly Ile Tyr Leu Phe Ser Leu Ser Leu Ser Asp Leu Leu
    50                  55                  60

Tyr Ala Leu Thr Leu Pro Leu Trp Ile Asn Tyr Thr Trp Asn Lys Asp
65                  70                  75                  80

Asn Trp Thr Phe Ser Pro Thr Leu Cys Lys Gly Ser Val Phe Phe Thr
                85                  90                  95
```

6

```
Tyr Met Asn Phe Tyr Ser Ser Thr Ala Phe Leu Thr Cys Ile Ala Leu
        100             105             110

Asp Arg Tyr Leu Ala Val Val Tyr Pro Leu Lys Phe Ser Phe Leu Arg
        115             120             125

Thr Arg Arg Phe Ala Phe Ile Thr Ser Leu Ser Ile Trp Ile Leu Glu
    130             135             140

Ser Phe Phe Asn Ser Met Leu Leu Trp Lys Asp Glu Thr Ser Val Glu
145             150             155             160

Tyr Cys Asp Ser Asp Lys Ser Asn Phe Thr Leu Cys Tyr Asp Lys Tyr
            165             170             175

Pro Leu Glu Lys Trp Gln Ile Asn Leu Asn Leu Phe Arg Thr Cys Met
        180             185             190

Gly Tyr Ala Ile Pro Leu Ile Thr Ile Met Ile Cys Asn His Lys Val
        195             200             205

Tyr Arg Ala Val Arg His Asn Gln Ala Thr Glu Asn Ser Glu Lys Arg
    210             215             220

Arg Ile Ile Lys Leu Leu Ala Ser Ile Thr Leu Thr Phe Val Leu Cys
225             230             235             240

Phe Thr Pro Phe His Val Met Val Leu Ile Arg Cys Val Leu Glu Arg
            245             250             255

Asp Met Asn Val Asn Asp Lys Ser Gly Trp Gln Thr Phe Thr Val Tyr
            260             265             270

Arg Val Thr Val Ala Leu Thr Ser Leu Asn Cys Val Ala Asp Pro Ile
        275             280             285

Leu Tyr Cys Phe Val Thr Glu Thr Gly Arg Ala Asp Met Trp Asn Ile
        290             295             300

Leu Lys Leu Cys Thr Arg Lys His Asn Arg His Gln Gly Lys Lys Arg
305             310             315             320

Asp Ile Leu Ser Val Ser Thr Arg Asp Ala Val Glu Leu Glu Ile Ile
            325             330             335

Asp
```

```
<210> 10
<211> 281
<212> PRT
<213> Mus musculus

<400> 10
Met Phe Ser Gly Leu Thr Leu Asn Cys Val Leu Leu Leu Leu Gln Leu
  1           5             10              15
```

7

```
Leu Leu Ala Arg Ser Leu Glu Asp Gly Tyr Lys Val Glu Val Gly Lys
            20                  25                  30

Asn Ala Tyr Leu Pro Cys Ser Tyr Thr Leu Pro Thr Ser Gly Thr Leu
            35                  40                  45

Val Pro Met Cys Trp Gly Lys Gly Phe Cys Pro Trp Ser Gln Cys Thr
        50                  55                  60

Asn Glu Leu Leu Arg Thr Asp Glu Arg Asn Val Thr Tyr Gln Lys Ser
65                  70                  75                  80

Ser Arg Tyr Gln Leu Lys Gly Asp Leu Asn Lys Gly Asp Val Ser Leu
                85                  90                  95

Ile Ile Lys Asn Val Thr Leu Asp Asp His Gly Thr Tyr Cys Cys Arg
            100                 105                 110

Ile Gln Phe Pro Gly Leu Met Asn Asp Lys Lys Leu Glu Leu Lys Leu
        115                 120                 125

Asp Ile Lys Ala Ala Lys Val Thr Pro Ala Gln Thr Ala His Gly Asp
    130                 135                 140

Ser Thr Thr Ala Ser Pro Arg Thr Leu Thr Thr Glu Arg Asn Gly Ser
145                 150                 155                 160

Glu Thr Gln Thr Leu Val Thr Leu His Asn Asn Asn Gly Thr Lys Ile
            165                 170                 175

Ser Thr Trp Ala Asp Glu Ile Lys Asp Ser Gly Glu Thr Ile Arg Thr
            180                 185                 190

Ala Ile His Ile Gly Val Gly Val Ser Ala Gly Leu Thr Leu Ala Leu
        195                 200                 205

Ile Ile Gly Val Leu Ile Leu Lys Trp Tyr Ser Cys Lys Lys Lys Lys
    210                 215                 220

Leu Ser Ser Leu Ser Leu Ile Thr Leu Ala Asn Leu Pro Pro Gly Gly
225                 230                 235                 240

Leu Ala Asn Ala Gly Ala Val Arg Ile Arg Ser Glu Glu Asn Ile Tyr
                245                 250                 255

Thr Ile Glu Glu Asn Val Tyr Glu Val Glu Asn Ser Asn Glu Tyr Tyr
            260                 265                 270

Cys Tyr Val Asn Ser Gln Gln Pro Ser
        275                 280
```

<210> 11
<211> 1257
<212> DNA
<213> Mus musculus

<220>

8

<221> CDS
<222> (22)..(1137)

<400> 11
ccgggtcgac ccacgcgtcc g atg aca ctg act gcc cac ctc tcc tac ttt   51
                        Met Thr Leu Thr Ala His Leu Ser Tyr Phe
                         1   5   10

ctg gtc ctg ttg tta gcg ggc caa ggc ctc agt gac tcc ctc ctc acc   99
Leu Val Leu Leu Leu Ala Gly Gln Gly Leu Ser Asp Ser Leu Leu Thr
                15   20   25

aag gat gca ggt ccc cgc cca ctg gag ctg aag gaa gtc ttc aag ctg   147
Lys Asp Ala Gly Pro Arg Pro Leu Glu Leu Lys Glu Val Phe Lys Leu
            30   35   40

ttc cag atc cgg ttc aac cgg agt tac tgg aac cca gca gag tac act   195
Phe Gln Ile Arg Phe Asn Arg Ser Tyr Trp Asn Pro Ala Glu Tyr Thr
            45   50   55

cgc cgt ctg agc atc ttt gcc cac aat ctg gct cag gct caa agg cta   243
Arg Arg Leu Ser Ile Phe Ala His Asn Leu Ala Gln Ala Gln Arg Leu
        60   65   70

cag caa gaa gac ttg ggt aca gct gag ttt gga gag act cca ttc agt   291
Gln Gln Glu Asp Leu Gly Thr Ala Glu Phe Gly Glu Thr Pro Phe Ser
75   80   85   90

gac ctc aca gag gag gag ttt ggc cag tta tac ggg cag gag agg tca   339
Asp Leu Thr Glu Glu Glu Phe Gly Gln Leu Tyr Gly Gln Glu Arg Ser
                95   100   105

cca gaa agg acc ccc aac atg acc aaa aag gta gag tct aac acg tgg   387
Pro Glu Arg Thr Pro Asn Met Thr Lys Lys Val Glu Ser Asn Thr Trp
            110   115   120

ggg gaa tct gtg ccc cgc acc tgt gac tgg cgt aaa gca aag aac atc   435
Gly Glu Ser Val Pro Arg Thr Cys Asp Trp Arg Lys Ala Lys Asn Ile
        125   130   135

atc tcg tcg gtc aag aac cag gga agc tgc aaa tgc tgc tgg gcc atg   483
Ile Ser Ser Val Lys Asn Gln Gly Ser Cys Lys Cys Cys Trp Ala Met
        140   145   150

gca gct gcc gac aac atc cag gct ctg tgg cgc atc aaa cac cag cag   531
Ala Ala Ala Asp Asn Ile Gln Ala Leu Trp Arg Ile Lys His Gln Gln
155   160   165   170

ttt gtg gac gtc tct gtg cag gag ctg ctg gac tgc gaa cgc tgt gga   579
Phe Val Asp Val Ser Val Gln Glu Leu Leu Asp Cys Glu Arg Cys Gly
                175   180   185

aat ggt tgc aat ggt ggc ttc gtg tgg gac gca tat cta act gtc ctc   627
Asn Gly Cys Asn Gly Gly Phe Val Trp Asp Ala Tyr Leu Thr Val Leu
                190   195   200

9

```
aac aac agt ggc ctg gcc agt gaa aag gat tat cca ttc cag ggg gac    675
Asn Asn Ser Gly Leu Ala Ser Glu Lys Asp Tyr Pro Phe Gln Gly Asp
        205             210             215

aga aag cct cac aga tgc cta gcc aag aag tac aag aag gtg gcc tgg    723
Arg Lys Pro His Arg Cys Leu Ala Lys Lys Tyr Lys Lys Val Ala Trp
    220             225             230

atc cag gat ttc acc atg ttg tcc aat aat gag cag gca att gcc cac    771
Ile Gln Asp Phe Thr Met Leu Ser Asn Asn Glu Gln Ala Ile Ala His
235             240             245             250

tac ctg gcc gtg cat gga cct atc acc gtg acc atc aac atg aaa cta    819
Tyr Leu Ala Val His Gly Pro Ile Thr Val Thr Ile Asn Met Lys Leu
            255             260             265

ctc cag cat tac cag aag ggt gtc atc aag gct aca ccc agc tcc tgt    867
Leu Gln His Tyr Gln Lys Gly Val Ile Lys Ala Thr Pro Ser Ser Cys
            270             275             280

gac cct cgg caa gtg gac cac tct gtc ttg ctg gtg ggc ttt ggc aag    915
Asp Pro Arg Gln Val Asp His Ser Val Leu Leu Val Gly Phe Gly Lys
        285             290             295

gag aaa gag ggc atg cag aca ggg aca gtc ttg tcc cat tct cga aaa    963
Glu Lys Glu Gly Met Gln Thr Gly Thr Val Leu Ser His Ser Arg Lys
    300             305             310

cgt cgc cac tcc tcc cca tac tgg atc ctg aag aac tcc tgg gga gct   1011
Arg Arg His Ser Ser Pro Tyr Trp Ile Leu Lys Asn Ser Trp Gly Ala
315             320             325             330

cac tgg ggc gag aag ggt tac ttc agg ctg tat cgg gga aac aac acc   1059
His Trp Gly Glu Lys Gly Tyr Phe Arg Leu Tyr Arg Gly Asn Asn Thr
            335             340             345

tgt gga gtc acc aag tat ccc ttc aca gct caa gtg gac tca cca gta   1107
Cys Gly Val Thr Lys Tyr Pro Phe Thr Ala Gln Val Asp Ser Pro Val
            350             355             360

aag aag gca cgg acc tct tgt cct ccc tga aggcagcagv cactcttctg    1157
Lys Lys Ala Arg Thr Ser Cys Pro Pro
        365             370

cttctcccac atggccactg cccttgtca gccctgccca catcctctct gtatggcttc 1217

ataaaccaag actgctccgt gaaaaaaaaa aaaaaaaaaa                       1257


<210> 12
<211> 371
<212> PRT
<213> Mus musculus

<400> 12
Met Thr Leu Thr Ala His Leu Ser Tyr Phe Leu Val Leu Leu Leu Ala
  1               5                  10                  15
```

```
Gly Gln Gly Leu Ser Asp Ser Leu Leu Thr Lys Asp Ala Gly Pro Arg
            20          25              30

Pro Leu Glu Leu Lys Glu Val Phe Lys Leu Phe Gln Ile Arg Phe Asn
            35          40              45

Arg Ser Tyr Trp Asn Pro Ala Glu Tyr Thr Arg Arg Leu Ser Ile Phe
        50          55          60

Ala His Asn Leu Ala Gln Ala Gln Arg Leu Gln Gln Glu Asp Leu Gly
65              70          75              80

Thr Ala Glu Phe Gly Glu Thr Pro Phe Ser Asp Leu Thr Glu Glu Glu
            85          90              95

Phe Gly Gln Leu Tyr Gly Gln Glu Arg Ser Pro Glu Arg Thr Pro Asn
            100         105             110

Met Thr Lys Lys Val Glu Ser Asn Thr Trp Gly Glu Ser Val Pro Arg
        115         120             125

Thr Cys Asp Trp Arg Lys Ala Lys Asn Ile Ile Ser Ser Val Lys Asn
    130         135             140

Gln Gly Ser Cys Lys Cys Cys Trp Ala Met Ala Ala Ala Asp Asn Ile
145             150         155             160

Gln Ala Leu Trp Arg Ile Lys His Gln Gln Phe Val Asp Val·Ser Val
            165         170             175

Gln Glu Leu Leu Asp Cys Glu Arg Cys Gly Asn Gly Cys Asn Gly Gly
            180         185             190

Phe Val Trp Asp Ala Tyr Leu Thr Val Leu Asn Asn Ser Gly Leu Ala
        195         200             205

Ser Glu Lys Asp Tyr Pro Phe Gln Gly Asp Arg Lys Pro His Arg Cys
    210         215             220

Leu Ala Lys Lys Tyr Lys Lys Val Ala Trp Ile Gln Asp Phe Thr Met
225             230         235             240

Leu Ser Asn Asn Glu Gln Ala Ile Ala His Tyr Leu Ala Val His Gly
            245         250             255

Pro Ile Thr Val Thr Ile Asn Met Lys Leu Leu Gln His Tyr Gln Lys
        260         265             270

Gly Val Ile Lys Ala Thr Pro Ser Ser Cys Asp Pro Arg Gln Val Asp
        275         280             285

His Ser Val Leu Leu Val Gly Phe Gly Lys Glu Lys Glu Gly Met Gln
    290         295             300

Thr Gly Thr Val Leu Ser His Ser Arg Lys Arg Arg His Ser Ser Pro
305             310             315             320
```

11

```
Tyr Trp Ile Leu Lys Asn Ser Trp Gly Ala His Trp Gly Glu Lys Gly
                325                 330                 335

Tyr Phe Arg Leu Tyr Arg Gly Asn Asn Thr Cys Gly Val Thr Lys Tyr
            340                 345                 350

Pro Phe Thr Ala Gln Val Asp Ser Pro Val Lys Lys Ala Arg Thr Ser
        355                 360                 365

Cys Pro Pro
    370


<210> 13
<211> 130
<212> PRT
<213> Mus musculus

<400> 13
Met Arg Gln Lys Ala Val Ser Leu Phe Leu Cys Tyr Leu Leu Leu Phe
  1               5                  10                  15

Thr Cys Ser Gly Val Glu Ala Gly Lys Lys Lys Cys Ser Glu Ser Ser
            20                  25                  30

Asp Ser Gly Ser Gly Phe Trp Lys Ala Leu Thr Phe Met Ala Val Gly
        35                  40                  45

Gly Gly Leu Ala Val Ala Gly Leu Pro Ala Leu Gly Phe Thr Gly Ala
      50                  55                  60

Gly Ile Ala Ala Asn Ser Val Ala Ala Ser Leu Met Ser Trp Ser Ala
 65                  70                  75                  80

Ile Leu Asn Gly Gly Gly Val Pro Ala Gly Gly Leu Val Ala Thr Leu
                85                  90                  95

Gln Ser Leu Gly Ala Gly Gly Ser Ser Val Ile Thr Gly Asn Ile Gly
            100                 105                 110

Ala Leu Met Gly Tyr Ala Thr His Lys Tyr Leu Asp Ser Glu Glu Asp
        115                 120                 125

Glu Glu
    130


<210> 14
<211> 130
<212> PRT
<213> Mus musculus

<400> 14
Met Arg Gln Lys Ala Val Ser Val Phe Leu Cys Tyr Leu Leu Leu Phe
  1               5                  10                  15

Thr Cys Ser Gly Val Glu Ala Gly Lys Lys Lys Cys Ser Glu Ser Ser
            20                  25                  30
```

12

```
Asp Ser Gly Ser Gly Phe Trp Lys Ala Leu Thr Phe Met Ala Val Gly
        35                  40                  45

Gly Gly Leu Ala Val Ala Gly Leu Pro Ala Leu Gly Phe Thr Gly Ala
        50                  55                  60

Gly Ile Ala Ala Asn Ser Val Ala Ala Ser Leu Met Ser Trp Ser Ala
65                  70                  75                  80

Ile Leu Asn Gly Gly Gly Val Pro Ala Gly Gly Leu Val Ala Thr Leu
                85                  90                  95

Gln Ser Leu Gly Ala Gly Gly Ser Ser Val Val Ile Gly Asn Ile Gly
            100                 105                 110

Ala Leu Met Arg Tyr Ala Thr His Lys Tyr Leu Asp Ser Glu Glu Asp
            115                 120                 125

Glu Glu
    130


<210> 15
<211> 110
<212> PRT
<213> Mus musculus

<400> 15
Val Glu Ala Gly Lys Lys Lys Cys Ser Glu Ser Ser Asp Ser Gly Ser
1               5                   10                  15

Gly Phe Trp Lys Ala Leu Thr Phe Met Ala Val Gly Gly Gly Leu Ala
            20                  25                  30

Val Ala Gly Leu Pro Ala Leu Gly Phe Thr Gly Ala Gly Ile Ala Ala
            35                  40                  45

Asn Ser Val Ala Ala Ser Leu Met Ser Trp Ser Ala Ile Leu Asn Gly
        50                  55                  60

Gly Gly Val Pro Ala Gly Gly Leu Val Ala Thr Leu Gln Ser Leu Gly
65              70                  75                  80

Ala Gly Gly Ser Ser Val Val Ile Gly Asn Ile Gly Ala Leu Met Gly
                85                  90                  95

Tyr Ala Thr His Lys Tyr Leu Asp Ser Glu Glu Asp Glu Glu
            100                 105                 110


<210> 16
<211> 107
<212> PRT
<213> Mus musculus

<400> 16
Gly Lys Lys Lys Cys Ser Glu Ser Ser Asp Ser Gly Ser Gly Phe Trp
1               5                   10                  15
```

```
Lys Ala Leu Thr Phe Met Ala Val Gly Gly Gly Leu Ala Val Ala Gly
            20              25              30

Leu Pro Ala Leu Gly Phe Thr Gly Ala Gly Ile Ala Ala Asn Ser Val
            35              40              45

Ala Ala Ser Leu Met Ser Trp Ser Ala Ile Leu Asn Gly Gly Gly Val
        50              55              60

Pro Ala Gly Gly Leu Val Ala Thr Leu Gln Ser Leu Gly Ala Gly Gly
65              70              75              80

Ser Ser Val Val Ile Gly Asn Ile Gly Ala Leu Met Gly Tyr Ala Thr
                85              90              95

His Lys Tyr Leu Asp Ser Glu Glu Asp Glu Glu
            100             105
```

```
<210> 17
<211> 122
<212> PRT
<213> Mus musculus

<400> 17
Met Glu Ala Ser Ala Leu Thr Ser Ser Ala Val Thr Ser Val Ala Lys
1               5               10              15

Val Val Arg Val Ala Ser Gly Ser Ala Val Val Leu Pro Leu Ala Arg
            20              25              30

Ile Ala Thr Val Val Ile Gly Gly Val Val Ala Met Ala Ala Val Pro
            35              40              45

Met Val Leu Ser Ala Met Gly Phe Thr Ala Ala Gly Ile Ala Ser Ser
        50              55              60

Ser Ile Ala Ala Lys Met Met Ser Ala Ala Ala Ile Ala Asn Gly Gly
65              70              75              80

Gly Val Ala Ser Gly Ser Leu Val Gly Thr Leu Gln Ser Leu Gly Ala
                85              90              95

Thr Gly Leu Ser Gly Leu Thr Lys Phe Ile Leu Gly Ser Ile Gly Ser
            100             105             110

Ala Ile Ala Ala Val Ile Ala Arg Phe Tyr
            115             120
```

```
<210> 18
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer
```

14

<400> 18
ttgccataga gagacctc   18

<210> 19
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 19
tgctgtccaa ttatacagg   19

<210> 20
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 20
gaacacggca ttgtcactaa ct   22

<210> 21
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 21
cctcatagat gggcactgtg t   21

<210> 22
<211> 843
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 22
atgttttcag gtcttaccct caactgtgtc ctgctgctgc tgcaactact acttgcaagg 60

tcattggaag atggttataa ggttgaggtt ggtaaaaatg cctatctgcc ctgcagttac 120

actctaccta catctgggac acttgtgcct atgtgctggg gcaagggatt ctgtccttgg 180

tcacagtgta ccaatgagtt gctcagaact gatgaaagaa atgtgacata tcagaaatcc 240

agcagatacc agctaaaggg cgatctcaac aaaggagatg tgtctctgat cataaagaat 300

```
gtgactctgg atgaccatgg gacctactgc tgcaggatac agttccctgg tcttatgaat 360

gataaaaaat tagaactgaa attagacatc aaagcagcca aggtcactcc agctcagact 420

gcccatgggg actctactac agcttctcca agaaccctaa ccacggagag aaatggttca 480

gagacacaga cactggtgac cctccataat aacaatggaa caaaaatttc cacatgggct 540

gatgaaatta aggactctgg agaaacgatc agaactgcta tccacattgg agtgggagtc 600

tctgctgggt tgaccctggc acttatcatt ggtgtcttaa tccttaaatg gtattcctgt 660

aagaaaaaga agttatcgag tttgagcctt attacactgg ccaacttgcc tccaggaggg 720

ttggcaaatg caggagcagt caggattcgc tctgaggaaa atatctacac catcgaggag 780

aacgtatatg aagtggagaa ttcaaatgag tactactgct acgtcaacag ccagcagcca 840

tcc                                                                  843
```

```
<210> 23
<211> 2236
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (42)..(944)

<400> 23
cgctaacaga ggtgtcctct gacttttctt ctgcaagctc c atg ttt tca cat ctt  56
                                              Met Phe Ser His Leu
                                                1               5


ccc ttt gac tgt gtc ctg ctg ctg ctg ctg cta cta ctt aca agg tcc     104
Pro Phe Asp Cys Val Leu Leu Leu Leu Leu Leu Leu Leu Thr Arg Ser
            10              15              20


tca gaa gtg gaa tac aga gcg gag gtc ggt cag aat gcc tat ctg ccc     152
Ser Glu Val Glu Tyr Arg Ala Glu Val Gly Gln Asn Ala Tyr Leu Pro
            25              30              35


tgc ttc tac acc cca gcc gcc cca ggg aac ctc gtg ccc gtc tgc tgg     200
Cys Phe Tyr Thr Pro Ala Ala Pro Gly Asn Leu Val Pro Val Cys Trp
            40              45              50


ggc aaa gga gcc tgt cct gtg ttt gaa tgt ggc aac gtg gtg ctc agg     248
Gly Lys Gly Ala Cys Pro Val Phe Glu Cys Gly Asn Val Val Leu Arg
    55              60              65


act gat gaa agg gat gtg aat tat tgg aca tcc aga tac tgg cta aat     296
Thr Asp Glu Arg Asp Val Asn Tyr Trp Thr Ser Arg Tyr Trp Leu Asn
70              75              80              85


ggg gat ttc cgc aaa gga gat gtg tcc ctg acc ata gag aat gtg act     344
Gly Asp Phe Arg Lys Gly Asp Val Ser Leu Thr Ile Glu Asn Val Thr
            90              95              100
```

16

```
cta gca gac agt ggg atc tac tgc tgc cgg atc caa atc cca ggc ata   392
Leu Ala Asp Ser Gly Ile Tyr Cys Cys Arg Ile Gln Ile Pro Gly Ile
            105                 110                 115

atg aat gat gaa aaa ttt aac ctg aag ttg gtc atc aaa cca gcc aag   440
Met Asn Asp Glu Lys Phe Asn Leu Lys Leu Val Ile Lys Pro Ala Lys
            120                 125                 130

gtc acc cct gca ccg act ctg cag aga gac ttc act gca gcc ttt cca   488
Val Thr Pro Ala Pro Thr Leu Gln Arg Asp Phe Thr Ala Ala Phe Pro
            135                 140                 145

agg atg ctt acc acc agg gga cat ggc cca gca gag aca cag aca ctg   536
Arg Met Leu Thr Thr Arg Gly His Gly Pro Ala Glu Thr Gln Thr Leu
150                 155                 160                 165

ggg agc ctc cct gat ata aat cta aca caa ata tcc aca ttg gcc aat   584
Gly Ser Leu Pro Asp Ile Asn Leu Thr Gln Ile Ser Thr Leu Ala Asn
            170                 175                 180

gag tta cgg gac tct aga ttg gcc aat gac tta cgg gac tct gga gca   632
Glu Leu Arg Asp Ser Arg Leu Ala Asn Asp Leu Arg Asp Ser Gly Ala
            185                 190                 195

acc atc aga ata ggc atc tac atc gga gca ggg atc tgt gct ggg ctg   680
Thr Ile Arg Ile Gly Ile Tyr Ile Gly Ala Gly Ile Cys Ala Gly Leu
            200                 205                 210

gct ctg gct ctt atc ttc ggc gct tta att ttc aaa tgg tat tct cat   728
Ala Leu Ala Leu Ile Phe Gly Ala Leu Ile Phe Lys Trp Tyr Ser His
            215                 220                 225

agc aaa gag aag ata cag aat tta agc ctc atc tct ttg gcc aac ctc   776
Ser Lys Glu Lys Ile Gln Asn Leu Ser Leu Ile Ser Leu Ala Asn Leu
230                 235                 240                 245

cct ccc tca gga ttg gca aat gca gta gca gag gga att cgc tca gaa   824
Pro Pro Ser Gly Leu Ala Asn Ala Val Ala Glu Gly Ile Arg Ser Glu
            250                 255                 260

gaa aac atc tat acc att gaa gag aac gta tat gaa gtg gag gag ccc   872
Glu Asn Ile Tyr Thr Ile Glu Glu Asn Val Tyr Glu Val Glu Glu Pro
            265                 270                 275

aat gag tat tat tgc tat gtc agc agc agg cag caa ccc tca caa cct   920
Asn Glu Tyr Tyr Cys Tyr Val Ser Ser Arg Gln Gln Pro Ser Gln Pro
            280                 285                 290

ttg ggt tgt cgc ttt gca atg cca tagatccaac caccttattt ttgagcttgg   974
Leu Gly Cys Arg Phe Ala Met Pro
            295                 300

tgttttgtct ttttcagaaa ctatgagctg tgtcacctga ctggttttgg aggttctgtc 1034

cactgctatg gagcagagtt ttcccatttt cagaagataa tgactcacat gggaattgaa 1094

ctgggacctg cactgaactt aaacaggcat gtcattgcct ctgtatttaa gccaacagag 1154
```

17

ttacccaacc cagagactgt taatcatgga tgttagagct caaacgggct tttatataca 1214

ctaggaattc ttgacgtggg gtctctggag ctccaggaaa ttcgggcaca tcatatgtcc 1274

atgaaacttc agataaacta ggraaaactg ggtgctgagg tgaaagcata actttttttgg 1334

cacagaaagt ctaaaggggc cactgattttt caaagagatc tgtgatccct ttttgttttt 1394

tgtttttgag atggagtctt gctctgttgc ccaggctgga gtgcaatggc acaatctcgg 1454

ctcactgcaa gctccgcctc ctgggttcaa gcgattctcc tgcctcagcc tcctgagtgg 1514

ctgggattac aggcatgcac caccatgcċc agctaatttg ttgtattttt agtagagaca 1574

gggtttcacc atgttggcca gtgtggtctc aaactcctga cctcatgatt tgcctgcctc 1634

ggcctcccaa agcactggga ttacaggcgt gagccaccac atccagccag tgatccttaa 1694

aagattaaga gatgactgga ctaggtctac cttgatcttg aagattccct tggaatgttg 1754

agatttaggc ttatttgagc actacctgcc caactgtcag tgccagtgca tagcccttct 1814

tttgtctccc ttatgaagac tgccctgcag ggctgagatg tggcaggagc tcccagggaa 1874

aaaggaagtg catttgattg tgtgtgtattg gccaagtttt gcttgttgtg tgcttgaaag 1934

aaaatatctc tgaccaactt ctgtattcgt ggaccaaact gaagctatat ttttcacaga 1994

agaagaagca gtgacgggga cacaaattct gttgcctggt ggaaagaagg caaaggcctt 2054

cagcaatcta tattaccagc gctggatcct ttgacagaga gtggtcccta aacttaaatt 2114

tcaagacggt ataggcttga tctgtcttgc ttattgttgc cccctgcgcc tagcacaatt 2174

ctgacacaca attggaactt actaaaaatt ttttttttact gttaaaaaaa aaaaaaaaaa 2234

aa   2236

<210> 24
<211> 301
<212> PRT
<213> Homo sapiens

<400> 24
Met Phe Ser His Leu Pro Phe Asp Cys Val Leu Leu Leu Leu Leu
  1   5   10   15

Leu Leu Thr Arg Ser Ser Glu Val Glu Tyr Arg Ala Glu Val Gly Gln
           20   25   30

Asn Ala Tyr Leu Pro Cys Phe Tyr Thr Pro Ala Ala Pro Gly Asn Leu
       35   40   45

Val Pro Val Cys Trp Gly Lys Gly Ala Cys Pro Val Phe Glu Cys Gly
   50   55   60

```
Asn Val Val Leu Arg Thr Asp Glu Arg Asp Val Asn Tyr Trp Thr Ser
65              70              75              80

Arg Tyr Trp Leu Asn Gly Asp Phe Arg Lys Gly Asp Val Ser Leu Thr
                85              90              95

Ile Glu Asn Val Thr Leu Ala Asp Ser Gly Ile Tyr Cys Cys Arg Ile
        100             105             110

Gln Ile Pro Gly Ile Met Asn Asp Glu Lys Phe Asn Leu Lys Leu Val
        115             120             125

Ile Lys Pro Ala Lys Val Thr Pro Ala Pro Thr Leu Gln Arg Asp Phe
        130             135             140

Thr Ala Ala Phe Pro Arg Met Leu Thr Thr Arg Gly His Gly Pro Ala
145             150             155             160

Glu Thr Gln Thr Leu Gly Ser Leu Pro Asp Ile Asn Leu Thr Gln Ile
                165             170             175

Ser Thr Leu Ala Asn Glu Leu Arg Asp Ser Arg Leu Ala Asn Asp Leu
        180             185             190

Arg Asp Ser Gly Ala Thr Ile Arg Ile Gly Ile Tyr Ile Gly Ala Gly
        195             200             205

Ile Cys Ala Gly Leu Ala Leu Ala Leu Ile Phe Gly Ala Leu Ile Phe
        210             215             220

Lys Trp Tyr Ser His Ser Lys Glu Lys Ile Gln Asn Leu Ser Leu Ile
225             230             235             240

Ser Leu Ala Asn Leu Pro Pro Ser Gly Leu Ala Asn Ala Val Ala Glu
                245             250             255

Gly Ile Arg Ser Glu Glu Asn Ile Tyr Thr Ile Glu Glu Asn Val Tyr
        260             265             270

Glu Val Glu Glu Pro Asn Glu Tyr Tyr Cys Tyr Val Ser Ser Arg Gln
        275             280             285

Gln Pro Ser Gln Pro Leu Gly Cys Arg Phe Ala Met Pro
290             295             300
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<210> 25
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
      oligonucleotide

<400> 25
aaatttattc tcgaggaccc acgcgtccgg atttccc   37

<210> 26
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
     oligonucleotide oligonucleotide

<400> 26
ttaatttgga tccccagttc tgatcgtttc tccagagtc   39

<210> 27
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
     oligonucleotide

<400> 27
aaatttattc tcgagcgcta acagaggtgt cc   32

<210> 28
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
     oligonucleotide

<400> 28
ttaatttgga tcccctctga tggttgctcc agagtcccg   39

<210> 29
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
     oligonucleotide

<400> 29
ccgcgggtac cagtaaatcg tcctggggtg g   31

<210> 30
<211> 36
<212> DNA
<213> Artificial Sequence

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<220>
<223> Description of Artificial Sequence:
      oligonucleotide

<400> 30
aaataaagga tccctacatc cagcaactat gtagta                        36


<210> 31
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
      oligonucleotide

<400> 31
gcgcaattga ctagtgaccc acgcgtccgg atttc                         35


<210> 32
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
      oligonucleotide

<400> 32
gacgcggatc ctcaggatgg ctgctggctg                               30


<210> 33
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
      oligonucleotide

<400> 33
gaacacacta gtactatcct gtgccattgc catagaga                      38


<210> 34
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
      oligonucleotide

<400> 34
ggaatattgg gcccttggat cccaagtctg cacacctgca ctcc               44
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<210> 35
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
      oligonucleotide

<400> 35
gtaaatcgtc ctggggtctg g   21

<210> 36
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
      oligonucleotide

<400> 36
ccttctgata acacaagcat aaatc   25

<210> 37
<211> 903
<212> DNA
<213> Homo sapiens

<400> 37
atgttttcac atcttccctt tgactgtgtc ctgctgctgc tgctgctact acttacaagg 60

tcctcagaag tggaatacag agcggaggtc ggtcagaatg cctatctgcc ctgcttctac 120

accccagccg ccccagggaa cctcgtgccc gtctgctggg gcaaaggagc ctgtcctgtg 180

tttgaatgtg gcaacgtggt gctcaggact gatgaaaggg atgtgaatta ttggacatcc 240

agatactggc taaatgggga tttccgcaaa ggagatgtgt ccctgaccat agagaatgtg 300

actctagcag acagtgggat ctactgctgc cggatccaaa tcccaggcat aatgaatgat 360

gaaaaattta acctgaagtt ggtcatcaaa ccagccaagg tcacccctgc accgactctg 420

cagagagact tcactgcagc ctttccaagg atgcttacca ccagggggaca tggcccagca 480

gagacacaga cactggggag cctccctgat ataaatctaa cacaaatatc cacattggcc 540

aatgagttac gggactctag attggccaat gacttacggg actctggagc aaccatcaga 600

ataggcatct acatcggagc agggatctgt gctgggctgg ctctggctct tatcttcggc 660

gctttaattt tcaaatggta ttctcatagc aaagagaaga tacagaattt aagcctcatc 720

tctttggcca acctccctcc ctcaggattg gcaaatgcag tagcagaggg aattcgctca 780

gaagaaaaca tctataccat tgaagagaac gtatatgaag tggaggagcc caatgagtat 840

tattgctatg tcagcagcag gcagcaaccc tcacaacctt tgggttgtcg ctttgcaatg 900

cca   903

<210> 38
<211> 1704
<212> DNA
<213> Mus musculus

<220>
<221> CAAT_signal
<222> (1)..(1704)

<220>
<221> CDS
<222> (1)..(1701)

<400> 38
atg att gac aga cag aga atg gga ctt tgg gct ttg gca att ctg aca   48
Met Ile Asp Arg Gln Arg Met Gly Leu Trp Ala Leu Ala Ile Leu Thr
 1   5   10   15

ctt ccc atg tat ttg aca gtt acg gag ggc agt aaa tcg tcc tgg ggt   96
Leu Pro Met Tyr Leu Thr Val Thr Glu Gly Ser Lys Ser Ser Trp Gly
                 20   25   30

ctg gaa aat gag gct tta att gtg aga tgc ccc caa aga gga cgc tcg   144
Leu Glu Asn Glu Ala Leu Ile Val Arg Cys Pro Gln Arg Gly Arg Ser
             35   40   45

act tat cct gtg gaa tgg tat tac tca gat aca aat gaa agt att cct   192
Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp Thr Asn Glu Ser Ile Pro
         50   55   60

act caa aaa aga aat cgg atc ttt gtc tca aga gat cgt ctg aag ttt   240
Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe
 65   70   75   80

cta cca gcc aga gtg gaa gac tct ggg att tat gct tgt gtt atc aga   288
Leu Pro Ala Arg Val Glu Asp Ser Gly Ile Tyr Ala Cys Val Ile Arg
                     85   90   95

agc ccc aac ttg aat aag act gga tac ttg aat gtc acc ata cat aaa   336
Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu Asn Val Thr Ile His Lys
                100   105   110

aag ccg cca agc tgc aat atc cct gat tat ttg atg tac tcg aca gta   384
Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr Leu Met Tyr Ser Thr Val
            115   120   125

cgt gga tca gat aaa aat ttc aag ata acg tgt cca aca att gac ctg   432
Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr Cys Pro Thr Ile Asp Leu
        130   135   140

                                  23

```
tat aat tgg aca gca cct gtt cag tgg ttt aag aac tgc aaa gct ctc    480
Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu
145                 150                 155                 160

caa gag cca agg ttc agg gca cac agg tcc tac ttg ttc att gac aac    528
Gln Glu Pro Arg Phe Arg Ala His Arg Ser Tyr Leu Phe Ile Asp Asn
                165                 170                 175

gtg act cat gat gat gaa ggt gac tac act tgt caa ttc aca cac gcg    576
Val Thr His Asp Asp Glu Gly Asp Tyr Thr Cys Gln Phe Thr His Ala
                180                 185                 190

gag aat gga acc aac tac atc gtg acg gcc acc aga tca ttc aca gtt    624
Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val
                195                 200                 205

gaa gaa aaa ggc ttt tct atg ttt cca gta att aca aat cct cca tac    672
Glu Glu Lys Gly Phe Ser Met Phe Pro Val Ile Thr Asn Pro Pro Tyr
210                 215                 220

aac cac aca atg gaa gtg gaa ata gga aaa cca gca agt att gcc tgt    720
Asn His Thr Met Glu Val Glu Ile Gly Lys Pro Ala Ser Ile Ala Cys
225                 230                 235                 240

tca gct tgc ttt ggc aaa ggc tct cac ttc ttg gct gat gtc ctg tgg    768
Ser Ala Cys Phe Gly Lys Gly Ser His Phe Leu Ala Asp Val Leu Trp
                245                 250                 255

cag att aac aaa aca gta gtt gga aat ttt ggt gaa gca aga att caa    816
Gln Ile Asn Lys Thr Val Val Gly Asn Phe Gly Glu Ala Arg Ile Gln
                260                 265                 270

gaa gag gaa ggt cga aat gaa agt tcc agc aat gac atg gat tgt tta    864
Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser Asn Asp Met Asp Cys Leu
                275                 280                 285

acc tca gtg tta agg ata act ggt gtg aca gaa aag gac ctg tcc ctg    912
Thr Ser Val Leu Arg Ile Thr Gly Val Thr Glu Lys Asp Leu Ser Leu
290                 295                 300

gaa tat gac tgt ctg gcc ctg aac ctt cat ggc atg ata agg cac acc    960
Glu Tyr Asp Cys Leu Ala Leu Asn Leu His Gly Met Ile Arg His Thr
305                 310                 315                 320

ata agg ctg aga agg aaa caa cca att gat cac cga agc atc tac tac   1008
Ile Arg Leu Arg Arg Lys Gln Pro Ile Asp His Arg Ser Ile Tyr Tyr
                325                 330                 335

ata gtt gct gga tgt agt tta ttg cta·atg ttt atc aat gtc ttg gtg   1056
Ile Val Ala Gly Cys Ser Leu Leu Leu Met Phe Ile Asn Val Leu Val
                340                 345                 350

ata gtc tta aaa gtg ttc tgg att gag gtt gct ctg ttc tgg aga gat   1104
Ile Val Leu Lys Val Phe Trp Ile Glu Val Ala Leu Phe Trp Arg Asp
                355                 360                 365
```

```
ata gtg aca cct tac aaa acc cgg aac gat ggc aag ctc tac gat gcg      1152
Ile Val Thr Pro Tyr Lys Thr Arg Asn Asp Gly Lys Leu Tyr Asp Ala
    370             375             380

tac atc att tac cct cgg gtc ttc cgg ggc agc gcg gcg gga acc cac      1200
Tyr Ile Ile Tyr Pro Arg Val Phe Arg Gly Ser Ala Ala Gly Thr His
385             390             395             400

tct gtg gag tac ttt gtt cac cac act ctg ccc gac gtt ctt gaa aat      1248
Ser Val Glu Tyr Phe Val His His Thr Leu Pro Asp Val Leu Glu Asn
            405             410             415

aaa tgt ggc tac aaa ttg tgc att tat ggg aga gac ctg tta cct ggg      1296
Lys Cys Gly Tyr Lys Leu Cys Ile Tyr Gly Arg Asp Leu Leu Pro Gly
            420             425             430

caa gat gca gcc acc gtg gtg gaa agc agt atc cag aat agc aga aga      1344
Gln Asp Ala Ala Thr Val Val Glu Ser Ser Ile Gln Asn Ser Arg Arg
            435             440             445

cag gtg ttt gtt ctg gcc cct cac atg atg cac agc aag gaa ttt gcc      1392
Gln Val Phe Val Leu Ala Pro His Met Met His Ser Lys Glu Phe Ala
    450             455             460

tac gag cag gag att gct ctg cac agc gcc ctc atc cag aac aac tcc      1440
Tyr Glu Gln Glu Ile Ala Leu His Ser Ala Leu Ile Gln Asn Asn Ser
465             470             475             480

aag gtg att ctt att gaa atg gag cct ctg ggt gag gca agc cga cta      1488
Lys Val Ile Leu Ile Glu Met Glu Pro Leu Gly Glu Ala Ser Arg Leu
            485             490             495

cag gtt ggg gac ctg caa gat tct ctc cag cat ctt gtg aaa att cag      1536
Gln Val Gly Asp Leu Gln Asp Ser Leu Gln His Leu Val Lys Ile Gln
            500             505             510

ggg acc atc aag tgg agg gaa gat cat gtg gcc gac aag cag tct cta      1584
Gly Thr Ile Lys Trp Arg Glu Asp His Val Ala Asp Lys Gln Ser Leu
            515             520             525

agt tcc aaa ttc tgg aag cat gtg agg tac caa atg cca gtg cca gaa      1632
Ser Ser Lys Phe Trp Lys His Val Arg Tyr Gln Met Pro Val Pro Glu
    530             535             540

aga gcc tcc aag acg gca tct gtt gcg gct ccg ttg agt ggc aag gca      1680
Arg Ala Ser Lys Thr Ala Ser Val Ala Ala Pro Leu Ser Gly Lys Ala
545             550             555             560

tgc tta gac ctg aaa cac ttt tga                                      1704
Cys Leu Asp Leu Lys His Phe
            565
```

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<210> 39
<211> 567
<212> PRT
<213> Mus musculus
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<400> 39

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Met Ile Asp Arg Gln Arg Met Gly Leu Trp Ala Leu Ala Ile Leu Thr
 1               5                   10                  15

Leu Pro Met Tyr Leu Thr Val Thr Glu Gly Ser Lys Ser Ser Trp Gly
                20                  25                  30

Leu Glu Asn Glu Ala Leu Ile Val Arg Cys Pro Gln Arg Gly Arg Ser
            35                  40                  45

Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp Thr Asn Glu Ser Ile Pro
        50                  55                  60

Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe
65                  70                  75                  80

Leu Pro Ala Arg Val Glu Asp Ser Gly Ile Tyr Ala Cys Val Ile Arg
                85                  90                  95

Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu Asn Val Thr Ile His Lys
            100                 105                 110

Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr Leu Met Tyr Ser Thr Val
        115                 120                 125

Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr Cys Pro Thr Ile Asp Leu
    130                 135                 140

Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu
145                 150                 155                 160

Gln Glu Pro Arg Phe Arg Ala His Arg Ser Tyr Leu Phe Ile Asp Asn
    .           165                 170                 175

Val Thr His Asp Asp Glu Gly Asp Tyr Thr Cys Gln Phe Thr His Ala
            180                 185                 190

Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val
            195                 200                 205

Glu Glu Lys Gly Phe Ser Met Phe Pro Val Ile Thr Asn Pro Pro Tyr
    210                 215                 220

Asn His Thr Met Glu Val Glu Ile Gly Lys Pro Ala Ser Ile Ala Cys
225                 230                 235                 240

Ser Ala Cys Phe Gly Lys Gly Ser His Phe Leu Ala Asp Val Leu Trp
                245                 250                 255

Gln Ile Asn Lys Thr Val Val Gly Asn Phe Gly Glu Ala Arg Ile Gln
            260                 265                 270

Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser Asn Asp Met Asp Cys Leu
        275                 280                 285

Thr Ser Val Leu Arg Ile Thr Gly Val Thr Glu Lys Asp Leu Ser Leu
    290                 295                 300
```

26

```
Glu Tyr Asp Cys Leu Ala Leu Asn Leu His Gly Met Ile Arg His Thr
305             310              315             320

Ile Arg Leu Arg Arg Lys Gln Pro Ile Asp His Arg Ser Ile Tyr Tyr
                325              330             335

Ile Val Ala Gly Cys Ser Leu Leu Leu Met Phe Ile Asn Val Leu Val
            340              345             350

Ile Val Leu Lys Val Phe Trp Ile Glu Val Ala Leu Phe Trp Arg Asp
            355              360             365

Ile Val Thr Pro Tyr Lys Thr Arg Asn Asp Gly Lys Leu Tyr Asp Ala
        370              375             380

Tyr Ile Ile Tyr Pro Arg Val Phe Arg Gly Ser Ala Ala Gly Thr His
385             390              395             400

Ser Val Glu Tyr Phe Val His His Thr Leu Pro Asp Val Leu Glu Asn
            405              410             415

Lys Cys Gly Tyr Lys Leu Cys Ile Tyr Gly Arg Asp Leu Leu Pro Gly
            420              425             430

Gln Asp Ala Ala Thr Val Val Glu Ser Ser Ile Gln Asn Ser Arg Arg
        435              440             445

Gln Val Phe Val Leu Ala Pro His Met Met His Ser Lys Glu Phe Ala
    450              455              460

Tyr Glu Gln Glu Ile Ala Leu His Ser Ala Leu Ile Gln Asn Asn Ser
465             470              475             480

Lys Val Ile Leu Ile Glu Met Glu Pro Leu Gly Glu Ala Ser Arg Leu
            485              490             495

Gln Val Gly Asp Leu Gln Asp Ser Leu Gln His Leu Val Lys Ile Gln
        500              505             510

Gly Thr Ile Lys Trp Arg Glu Asp His Val Ala Asp Lys Gln Ser Leu
        515              520             525

Ser Ser Lys Phe Trp Lys His Val Arg Tyr Gln Met Pro Val Pro Glu
    530              535              540

Arg Ala Ser Lys Thr Ala Ser Val Ala Ala Pro Leu Ser Gly Lys Ala
545             550              555             560

Cys Leu Asp Leu Lys His Phe
                565
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<210> 40
<211> 1029
<212> DNA
<213> Mus musculus

<220>
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27

<221> CDS
<222> (1)..(1026)

<400> 40
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atg att gac aga cag aga atg gga ctt tgg gct ttg gca att ctg aca    48
Met Ile Asp Arg Gln Arg Met Gly Leu Trp Ala Leu Ala Ile Leu Thr
1               5                   10                  15

ctt ccc atg tat ttg aca gtt acg gag ggc agt aaa tcg tcc tgg ggt    96
Leu Pro Met Tyr Leu Thr Val Thr Glu Gly Ser Lys Ser Ser Trp Gly
                20                  25                  30

ctg gaa aat gag gct tta att gtg aga tgc ccc caa aga gga cgc tcg   144
Leu Glu Asn Glu Ala Leu Ile Val Arg Cys Pro Gln Arg Gly Arg Ser
            35                  40                  45

act tat cct gtg gaa tgg tat tac tca gat aca aat gaa agt att cct   192
Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp Thr Asn Glu Ser Ile Pro
        50                  55                  60

act caa aaa aga aat cgg atc ttt gtc tca aga gat cgt ctg aag ttt   240
Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe
65                  70                  75                  80

cta cca gcc aga gtg gaa gac tct ggg att tat gct tgt gtt atc aga   288
Leu Pro Ala Arg Val Glu Asp Ser Gly Ile Tyr Ala Cys Val Ile Arg
                85                  90                  95

agc ccc aac ttg aat aag act gga tac ttg aat gtc acc ata cat aaa   336
Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu Asn Val Thr Ile His Lys
            100                 105                 110

aag ccg cca agc tgc aat atc cct gat tat ttg atg tac tcg aca gta   384
Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr Leu Met Tyr Ser Thr Val
            115                 120                 125

cgt gga tca gat aaa aat ttc aag ata acg tgt cca aca att gac ctg   432
Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr Cys Pro Thr Ile Asp Leu
        130                 135                 140

tat aat tgg aca gca cct gtt cag tgg ttt aag aac tgc aaa gct ctc   480
Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu
145                 150                 155                 160

caa gag cca agg ttc agg gca cac agg tcc tac ttg ttc att gac aac   528
Gln Glu Pro Arg Phe Arg Ala His Arg Ser Tyr Leu Phe Ile Asp Asn
                165                 170                 175

gtg act cat gat gat gaa ggt gac tac act tgt caa ttc aca cac gcg   576
Val Thr His Asp Asp Glu Gly Asp Tyr Thr Cys Gln Phe Thr His Ala
            180                 185                 190

gag aat gga acc aac tac atc gtg acg gcc acc aga tca ttc aca gtt   624
Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val
            195                 200                 205
```

```
gaa gaa aaa ggc ttt tct atg ttt cca gta att aca aat cct cca tac     672
Glu Glu Lys Gly Phe Ser Met Phe Pro Val Ile Thr Asn Pro Pro Tyr
    210             215             220

aac cac aca atg gaa gtg gaa ata gga aaa cca gca agt att gcc tgt     720
Asn His Thr Met Glu Val Glu Ile Gly Lys Pro Ala Ser Ile Ala Cys
225             230             235             240

tca gct tgc ttt ggc aaa ggc tct cac ttc ttg gct gat gtc ctg tgg    768
Ser Ala Cys Phe Gly Lys Gly Ser His Phe Leu Ala Asp Val Leu Trp
                245             250             255

cag att aac aaa aca gta gtt gga aat ttt ggt gaa gca aga att caa     816
Gln Ile Asn Lys Thr Val Val Gly Asn Phe Gly Glu Ala Arg Ile Gln
                260             265             270

gaa gag gaa ggt cga aat gaa agt tcc agc aat gac atg gat tgt tta     864
Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser Asn Asp Met Asp Cys Leu
        275             280             285

acc tca gtg tta agg ata act ggt gtg aca gaa aag gac ctg tcc ctg     912
Thr Ser Val Leu Arg Ile Thr Gly Val Thr Glu Lys Asp Leu Ser Leu
        290             295             300

gaa tat gac tgt ctg gcc ctg aac ctt cat ggc atg ata agg cac acc     960
Glu Tyr Asp Cys Leu Ala Leu Asn Leu His Gly Met Ile Arg His Thr
305             310             315             320

ata agg ctg aga agg aaa caa cca att gat cac cga agc atc tac tac    1008
Ile Arg Leu Arg Arg Lys Gln Pro Ile Asp His Arg Ser Ile Tyr Tyr
                325             330             335

ata gtt gct gga tgt agt tga                                        1029
Ile Val Ala Gly Cys Ser
                340
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<210> 41
<211> 342
<212> PRT
<213> Mus musculus

<400> 41
Met Ile Asp Arg Gln Arg Met Gly Leu Trp Ala Leu Ala Ile Leu Thr
1               5               10              15

Leu Pro Met Tyr Leu Thr Val Thr Glu Gly Ser Lys Ser Ser Trp Gly
            20              25              30

Leu Glu Asn Glu Ala Leu Ile Val Arg Cys Pro Gln Arg Gly Arg Ser
        35              40              45

Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp Thr Asn Glu Ser Ile Pro
    50              55              60

Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe
65              70              75              80
```

29

Leu Pro Ala Arg Val Glu Asp Ser Gly Ile Tyr Ala Cys Val Ile Arg
              85   90   95

Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu Asn Val Thr Ile His Lys
              100   105   110

Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr Leu Met Tyr Ser Thr Val
              115·   120   125

Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr Cys Pro Thr Ile Asp Leu
      130   135   140

Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu
145   150   155   160

Gln Glu Pro Arg Phe Arg Ala His Arg Ser Tyr Leu Phe Ile Asp Asn
              165   170   175

Val Thr His Asp Asp Glu Gly Asp Tyr Thr Cys Gln Phe Thr His Ala
              180   185   190

Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val
              195   200   205

Glu Glu Lys Gly Phe Ser Met Phe Pro Val Ile Thr Asn Pro Pro Tyr
      210   215   220

Asn His Thr Met Glu Val Glu Ile Gly Lys Pro Ala Ser Ile Ala Cys
225   230   235   240

Ser Ala Cys Phe Gly Lys Gly Ser His Phe Leu Ala Asp Val Leu Trp
              245   250   255

Gln Ile Asn Lys Thr Val Val Gly Asn Phe Gly Glu Ala Arg Ile Gln
              260   265   270

Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser Asn Asp Met Asp Cys Leu
      275   280   285

Thr Ser Val Leu Arg Ile Thr Gly Val Thr Glu Lys Asp Leu Ser Leu
290   295   300

Glu Tyr Asp Cys Leu Ala Leu Asn Leu His Gly Met Ile Arg His Thr
305   310   315   320

Ile Arg Leu Arg Arg Lys Gln Pro Ile Asp His Arg Ser Ile Tyr Tyr
              325   330   335

Ile Val Ala Gly Cys Ser
              340

<210> 42
<211> 606
<212> DNA
<213> Mus musculus

<220>

30

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<222> (1)..(603)

<400> 42

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Arg Asp Ile Val Thr Pro Tyr Lys Thr Arg Asn Asp Gly Lys Leu Tyr
 1               5                  10                  15

gat gcg tac atc att tac cct cgg gtc ttc cgg ggc agc gcg gcg gga    96
Asp Ala Tyr Ile Ile Tyr Pro Arg Val Phe Arg Gly Ser Ala Ala Gly
                20                  25                  30

acc cac tct gtg gag tac ttt gtt cac cac act ctg ccc gac gtt ctt   144
Thr His Ser Val Glu Tyr Phe Val His His Thr Leu Pro Asp Val Leu
            35                  40                  45

gaa aat aaa tgt ggc tac aaa ttg tgc att tat ggg aga gac ctg tta   192
Glu Asn Lys Cys Gly Tyr Lys Leu Cys Ile Tyr Gly Arg Asp Leu Leu
        50                  55                  60

cct ggg caa gat gca gcc acc gtg gtg gaa agc agt atc cag aat agc   240
Pro Gly Gln Asp Ala Ala Thr Val Val Glu Ser Ser Ile Gln Asn Ser
 65                  70                  75                  80

aga aga cag gtg ttt gtt ctg gcc cct cac atg atg cac agc aag gaa   288
Arg Arg Gln Val Phe Val Leu Ala Pro His Met Met His Ser Lys Glu
                85                  90                  95

ttt gcc tac gag cag gag att gct ctg cac agc gcc ctc atc cag aac   336
Phe Ala Tyr Glu Gln Glu Ile Ala Leu His Ser Ala Leu Ile Gln Asn
                100                 105                 110

aac tcc aag gtg att ctt att gaa atg gag cct ctg ggt gag gca agc   384
Asn Ser Lys Val Ile Leu Ile Glu Met Glu Pro Leu Gly Glu Ala Ser
            115                 120                 125

cga cta cag gtt ggg gac ctg caa gat tct ctc cag cat ctt gtg aaa   432
Arg Leu Gln Val Gly Asp Leu Gln Asp Ser Leu Gln His Leu Val Lys
        130                 135                 140

att cag ggg acc atc aag tgg agg gaa gat cat gtg gcc gac aag cag   480
Ile Gln Gly Thr Ile Lys Trp Arg Glu Asp His Val Ala Asp Lys Gln
145                 150                 155                 160

tct cta agt tcc aaa ttc tgg aag cat gtg agg tac caa atg cca gtg   528
Ser Leu Ser Ser Lys Phe Trp Lys His Val Arg Tyr Gln Met Pro Val
                165                 170                 175

cca gaa aga gcc tcc aag acg gca tct gtt gcg gct ccg ttg agt ggc   576
Pro Glu Arg Ala Ser Lys Thr Ala Ser Val Ala Ala Pro Leu Ser Gly
                180                 185                 190

aag gca tgc tta gac ctg aaa cac ttt tga                           606
Lys Ala Cys Leu Asp Leu Lys His Phe
        195                 200
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<212> PRT
<213> Mus musculus

<400> 43
Arg Asp Ile Val Thr Pro Tyr Lys Thr Arg Asn Asp Gly Lys Leu Tyr
 1               5                  10                  15

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              20              25                  30

Thr His Ser Val Glu Tyr Phe Val His His Thr Leu Pro Asp Val Leu
          35              40                  45

Glu Asn Lys Cys Gly Tyr Lys Leu Cys Ile Tyr Gly Arg Asp Leu Leu
      50              55              60

Pro Gly Gln Asp Ala Ala Thr Val Val Glu Ser Ser Ile Gln Asn Ser
65              70                  75                  80

Arg Arg Gln Val Phe Val Leu Ala Pro His Met Met His Ser Lys Glu
              85              90                  95

Phe Ala Tyr Glu Gln Glu Ile Ala Leu His Ser Ala Leu Ile Gln Asn
              100             105                 110

Asn Ser Lys Val Ile Leu Ile Glu Met Glu Pro Leu Gly Glu Ala Ser
          115             120                 125

Arg Leu Gln Val Gly Asp Leu Gln Asp Ser Leu Gln His Leu Val Lys
      130             135                 140

Ile Gln Gly Thr Ile Lys Trp Arg Glu Asp His Val Ala Asp Lys Gln
145             150                 155                 160

Ser Leu Ser Ser Lys Phe Trp Lys His Val Arg Tyr Gln Met Pro Val
              165             170                 175

Pro Glu Arg Ala Ser Lys Thr Ala Ser Val Ala Ala Pro Leu Ser Gly
              180             185                 190

Lys Ala Cys Leu Asp Leu Lys His Phe
          195             200


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<211> 1357
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<213> Homo sapiens

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                                                  Met Gly Phe
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tgg atc tta gca att ctc aca att ctc atg tat tcc aca gca gca aag    103
Trp Ile Leu Ala Ile Leu Thr Ile Leu Met Tyr Ser Thr Ala Ala Lys
     5               10              15

ttt agt aaa caa tca tgg ggc ctg gaa aat gag gct tta att gta aga    151
Phe Ser Lys Gln Ser Trp Gly Leu Glu Asn Glu Ala Leu Ile Val Arg
 20              25              30                      35

tgt cct aga caa gga aaa cct agt tac acc gtg gat tgg tat tac tca    199
Cys Pro Arg Gln Gly Lys Pro Ser Tyr Thr Val Asp Trp Tyr Tyr Ser
             40              45              50

caa aca aac aaa agt att ccc act cag gaa aga aat cgt gtg ttt gcc    247
Gln Thr Asn Lys Ser Ile Pro Thr Gln Glu Arg Asn Arg Val Phe Ala
             55              60              65

tca ggc caa ctt ctg aag ttt cta cca gct gaa gtt gct gat tct ggt    295
Ser Gly Gln Leu Leu Lys Phe Leu Pro Ala Glu Val Ala Asp Ser Gly
         70              75              80

att tat acc tgt att gtc aga agt ccc aca ttc aat agg act gga tat    343
Ile Tyr Thr Cys Ile Val Arg Ser Pro Thr Phe Asn Arg Thr Gly Tyr
         85              90              95

gcg aat gtc acc ata tat aaa aaa caa tca gat tgc aat gtt cca gat    391
Ala Asn Val Thr Ile Tyr Lys Lys Gln Ser Asp Cys Asn Val Pro Asp
100              105             110             115

tat ttg atg tat tca aca gta tct gga tca gaa aaa aat tcc aaa att    439
Tyr Leu Met Tyr Ser Thr Val Ser Gly Ser Glu Lys Asn Ser Lys Ile
             120             125             130

tat tgt cct acc att gac ctc tac aac tgg aca gca cct ctt gag tgg    487
Tyr Cys Pro Thr Ile Asp Leu Tyr Asn Trp Thr Ala Pro Leu Glu Trp
             135             140             145

ttt aag aat tgt cag gct ctt caa gga tca agg tac agg gcg cac aag    535
Phe Lys Asn Cys Gln Ala Leu Gln Gly Ser Arg Tyr Arg Ala His Lys
             150             155             160

tca ttt ttg gtc att gat aat gtg atg act gag gac gca ggt gat tac    583
Ser Phe Leu Val Ile Asp Asn Val Met Thr Glu Asp Ala Gly Asp Tyr
             165             170             175

acc tgt aaa ttt ata cac aat gaa aat gga gcc aat tat agt gtg acg    631
Thr Cys Lys Phe Ile His Asn Glu Asn Gly Ala Asn Tyr Ser Val Thr
180              185             190             195

gcg acc agg tcc ttc acg gtc aag gat gag caa ggc ttt tct ctg ttt    679
Ala Thr Arg Ser Phe Thr Val Lys Asp Glu Gln Gly Phe Ser Leu Phe
             200             205             210

cca gta atc gga gcc cct gca caa aat gaa ata aag gaa gtg gaa att    727
Pro Val Ile Gly Ala Pro Ala Gln Asn Glu Ile Lys Glu Val Glu Ile
             215             220             225
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33

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gga aaa aac gca aac cta act tgc tct gct tgt ttt gga aaa ggc act    775
Gly Lys Asn Ala Asn Leu Thr Cys Ser Ala Cys Phe Gly Lys Gly Thr
        230                 235                 240

cag ttc ttg gct gcc gtc ctg tgg cag ctt aat gga aca aaa att aca    823
Gln Phe Leu Ala Ala Val Leu Trp Gln Leu Asn Gly Thr Lys Ile Thr
    245                 250                 255

gac ttt ggt gaa cca aga att caa caa gag gaa ggg caa aat caa agt    871
Asp Phe Gly Glu Pro Arg Ile Gln Gln Glu Glu Gly Gln Asn Gln Ser
260                 265                 270                 275

ttc agc aat ggg ctg gct tgt cta gac atg gtt tta aga ata gct gac    919
Phe Ser Asn Gly Leu Ala Cys Leu Asp Met Val Leu Arg Ile Ala Asp
                280                 285                 290

gtg aag gaa gag gat tta ttg ctg cag tac gac tgt ctg gcc ctg aat    967
Val Lys Glu Glu Asp Leu Leu Leu Gln Tyr Asp Cys Leu Ala Leu Asn
            295                 300                 305

ttg cat ggc ttg aga agg cac acc gta aga cta agt agg aaa aat cca   1015
Leu His Gly Leu Arg Arg His Thr Val Arg Leu Ser Arg Lys Asn Pro
            310                 315                 320

agt aag gag tgt ttc tgagactttg atcacctgaa ctttctctag caagtgtaag   1070
Ser Lys Glu Cys Phe
        325

cagaatggag tgtggttcca agagatccat caagacaatg ggaatggcct gtgccataaa 1130

atgtgcttct cttcttcggg atgttgtttg ctgtctgatc tttgtagact gttcctgttt 1190

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ttgtctagaa cactcagctg cttctttggt catccttgtt ttctaacttt atgaactccc 1310

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<211> 328
<212> PRT
<213> Homo sapiens

<400> 45
Met Gly Phe Trp Ile Leu Ala Ile Leu Thr Ile Leu Met Tyr Ser Thr
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Ala Ala Lys Phe Ser Lys Gln Ser Trp Gly Leu Glu Asn Glu Ala Leu
            20                 25                  30

Ile Val Arg Cys Pro Arg Gln Gly Lys Pro Ser Tyr Thr Val Asp Trp
        35                 40                  45

Tyr Tyr Ser Gln Thr Asn Lys Ser Ile Pro Thr Gln Glu Arg Asn Arg
    50                 55                  60
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Val Phe Ala Ser Gly Gln Leu Leu Lys Phe Leu Pro Ala Glu Val Ala
 65              70          75            80

Asp Ser Gly Ile Tyr Thr Cys Ile Val Arg Ser Pro Thr Phe Asn Arg
            85              90              95

Thr Gly Tyr Ala Asn Val Thr Ile Tyr Lys Lys Gln Ser Asp Cys Asn
            100             105             110

Val Pro Asp Tyr Leu Met Tyr Ser Thr Val Ser Gly Ser Glu Lys Asn
        115             120             125

Ser Lys Ile Tyr Cys Pro Thr Ile Asp Leu Tyr Asn Trp Thr Ala Pro
    130             135             140

Leu Glu Trp Phe Lys Asn Cys Gln Ala Leu Gln Gly Ser Arg Tyr Arg
145             150             155             160

Ala His Lys Ser Phe Leu Val Ile Asp Asn Val Met Thr Glu Asp Ala
            165             170             175

Gly Asp Tyr Thr Cys Lys Phe Ile His Asn Glu Asn Gly Ala Asn Tyr
            180             185             190

Ser Val Thr Ala Thr Arg Ser Phe Thr Val Lys Asp Glu Gln Gly Phe
        195             200             205

Ser Leu Phe Pro Val Ile Gly Ala Pro Ala Gln Asn Glu Ile Lys Glu
    210             215             220

Val Glu Ile Gly Lys Asn Ala Asn Leu Thr Cys Ser Ala Cys Phe Gly
225             230             235             240

Lys Gly Thr Gln Phe Leu Ala Ala Val Leu Trp Gln Leu Asn Gly Thr
            245             250             255

Lys Ile Thr Asp Phe Gly Glu Pro Arg Ile Gln Gln Glu Glu Gly Gln
            260             265             270

Asn Gln Ser Phe Ser Asn Gly Leu Ala Cys Leu Asp Met Val Leu Arg
        275             280             285

Ile Ala Asp Val Lys Glu Glu Asp Leu Leu Leu Gln Tyr Asp Cys Leu
    290             295             300

Ala Leu Asn Leu His Gly Leu Arg Arg His Thr Val Arg Leu Ser Arg
305             310             315             320

Lys Asn Pro Ser Lys Glu Cys Phe
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<400> 46

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gctctgttct gg   72

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<212> DNA
<213> Mus musculus

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agatgccccc aaagaggacg ctcgacttat cctgtggaat ggtattactc agatacaaat 180

gaaagtattc ctactcaaaa aagaaatcgg atctttgtct caagagatcg tctgaagttt 240

ctaccagcca gagtcgaaga ctctgggatt tatgcttgtg ttatcàgaag ccccaacttg 300

aataagactg gatacttgaa tgtcaccata cataaaaagc cgccaagctg caatatccct 360

gattatttga tgtactcgac agtacgtgga tcagataaaa atttcaagat aagctgtcca 420

acaattgacc tgtataattg gacagcacct gttcagtggt ttaagaactg caaagctctc 480

caagagccaa ggttcagggc acacaggtcc tacttgttca ttgacaacgt gactcatgat 540

gatgaaggtg actacacttg tcaattcaca cacgcggaga atggaaccaa ctacatcgtg 600

acggccacca gatcattcac agttgaagaa aaaggctttt ctatgtttcc agtaattaca 660

aatcctccat acaaccacac aatggaagtg gaaataggaa aaccagcaag tattgcctgt 720

tcagcttgct ttggcaaagg ctctcacttc ttggctgatg tcctgtggca gattaacaaa 780

acagtagttg gaaattttgg tgaagcaaga attcaagaag aggaaggtcg aaatgaaagt 840

tccagcaatg acatggattg tttaacctca gtgttaagga taactggtgt gacagaaaag 900

gacctgtccc tggaatatga ctgtctggcc ctgaaccttc atggcatgat aaggcacacc 960

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<211> 337
<212> PRT
<213> Mus musculus

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Leu Pro Met Tyr Leu Thr Val Thr Glu Gly Ser Lys Ser Ser Trp Gly
            20   25   30

36

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Leu Glu Asn Glu Ala Leu Ile Val Arg Cys Pro Gln Arg Gly Arg Ser
        35              40              45

Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp Thr Asn Glu Ser Ile Pro
    50              55              60

Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe
65              70              75              80

Leu Pro Ala Arg Val Glu Asp Ser Gly Ile Tyr Ala Cys Val Ile Arg
            85              90              95

Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu Asn Val Thr Ile His Lys
            100             105             110

Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr Leu Met Tyr Ser Thr Val
        115             120             125

Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr Cys Pro Thr Ile Asp Leu
    130             135             140

Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu
145             150             155             160

Gln Glu Pro Arg Phe Arg Ala His Arg Ser Tyr Leu Phe Ile Asp Asn
            165             170             175

Val Thr His Asp Asp Glu Gly Asp Tyr Thr Cys Gln Phe Thr His Ala
        180             185             190

Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val
        195             200             205

Glu Glu Lys Gly Phe Ser Met Phe Pro Val Ile Thr Asn Pro Pro Tyr
    210             215             220

Asn His Thr Met Glu Val Glu Ile Gly Lys Pro Ala Ser Ile Ala Cys
225             230             235             240

Ser Ala Cys Phe Gly Lys Gly Ser His Phe Leu Ala Asp Val Leu Trp
            245             250             255

Gln Ile Asn Lys Thr Val Val Gly Asn Phe Gly Glu Ala Arg Ile Gln
            260             265             270

Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser Asn Asp Met Asp Cys Leu
    275             280             285

Thr Ser Val Leu Arg Ile Thr Gly Val Thr Glu Lys Asp Leu Ser Leu
    290             295             300

Glu Tyr Asp Cys Leu Ala Leu Asn Leu His Gly Met Ile Arg His Thr
305             310             315             320

Ile Arg Leu Arg Arg Lys Gln Pro Ser Lys Glu Cys Pro Ser His Ile
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Ala
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37

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<211> 337
<212> PRT
<213> Mus musculus

<400> 49
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 1               5                  10                  15

Leu Pro Met Tyr Leu Thr Val Thr Glu Gly Ser Lys Ser Ser Trp Gly
               20                  25                  30

Leu Glu Asn Glu Ala Leu Ile Val Arg Cys Pro Gln Arg Gly Arg Ser
           35                  40                  45

Thr Tyr Pro Val Glu Trp Tyr Tyr Ser Asp Thr Asn Glu Ser Ile Pro
       50                  55                  60

Thr Gln Lys Arg Asn Arg Ile Phe Val Ser Arg Asp Arg Leu Lys Phe
65                  70                  75                  80

Leu Pro Ala Arg Val Glu Asp Ser Gly Ile Tyr Ala Cys Val Ile Arg
                85                  90                  95

Ser Pro Asn Leu Asn Lys Thr Gly Tyr Leu Asn Val Thr Ile His Lys
           100                 105                 110

Lys Pro Pro Ser Cys Asn Ile Pro Asp Tyr Leu Met Tyr Ser Thr Val
       115                 120                 125

Arg Gly Ser Asp Lys Asn Phe Lys Ile Thr Cys Pro Thr Ile Asp Leu
   130                 135                 140

Tyr Asn Trp Thr Ala Pro Val Gln Trp Phe Lys Asn Cys Lys Ala Leu
145                 150                 155                 160

Gln Glu Pro Arg Phe Arg Ala His Arg Ser Tyr Leu Phe Ile Asp Asn
               165                 170                 175

Val Thr His Asp Asp Glu Gly Asp Tyr Thr Cys Gln Phe Thr His Ala
           180                 185                 190

Glu Asn Gly Thr Asn Tyr Ile Val Thr Ala Thr Arg Ser Phe Thr Val
           195                 200                 205

Glu Glu Lys Gly Phe Ser Met Phe Pro Val Ile Thr Asn Pro Pro Tyr
       210                 215                 220

Asn His Thr Met Glu Val Glu Ile Gly Lys Pro Ala Ser Ile Ala Cys
225                 230                 235                 240

Ser Ala Cys Phe Gly Lys Gly Ser His Phe Leu Ala Asp Val Leu Trp
               245                 250                 255
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Gln Ile Asn Lys Thr Val Val Gly Asn Phe Gly Glu Ala Arg Ile Gln
            260             265                 270

Glu Glu Glu Gly Arg Asn Glu Ser Ser Ser Asn Asp Met Asp Cys Leu
            275             280                 285

Thr Ser Val Leu Arg Ile Thr Gly Val Thr Glu Lys Asp Leu Ser Leu
    290             295                 300

Glu Tyr Asp Cys Leu Ala Leu Asn Leu His Gly Met Ile Arg His Thr
305             310                 315                     320

Ile Arg Leu Arg Arg Lys Gln Pro Ser Lys Glu Cys Pro Ser His Ile
            325             330                 335

Ala
```

INTERNATIONAL SEARCH REPORT

A.	CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12Q 1/66; C07H 19/00, 21/00, 21/02, 21/04
US CL :435/8; 536/22.1, 23.1, 23.4

According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/8; 536/22.1, 23.1, 23.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

biosis, caplus, caba, embase, lifesci, medline, scisearch, uspatfull, japio

C.	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,840,691 A (FURCHT et al) 24 November 1998, col. 9, lines 10-65, col. 19, line 51 to col. 21, line 2.	1, 4-11, 13, 14
Y	US 5,591,719 A (FURCHT et al) 07 January 1997, col. 5, line 45 to col. 8, line 34.	1, 4-11, 13, 14
Y	US 5,721,351 A (LEVINSON) 24 February 1998, col. 13, line 10 to col. 80, line 37.	1, 4-11, 13, 14

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
	2 3 AUG 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer RODNEY P. SCHARTZ, PH.D.
Facsimile No.	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

   Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
   1, 4-11, 13, 14

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

   ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)*

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1, 4-11, 13, and 14, drawn to first method of treatment of ischemic disorder by giving a 200 gene product.
Group II, claims 2, 4-12, 15, and 16, drawn to second method of treatment of ischemic disorder by gene therapy.
Group III, claims 3-11 and 17-21, drawn to third method of treatment of ischemic disorder by giving an antibody.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: each invention is drawn to a separate inventive concept. Invention I is drawn to a gene product, i.e., proteins. Invention II is drawn to gene therapy, i.e., administration of DNA. Invention III is drawn to passive immunity, i.e., administration of antibody.